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# Organohalogen concentrations and a gross and histologic assessment of multiple organ systems in East Greenland polar bears *(Ursus maritimus)*

***PhD thesis***

*Christian Sonne*

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***2004***

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Abstract: To investigate the relation between biological parameters, not earlier investigated in the polar bear, and organohalogen pollution in East Greenland polar bears, we initiated a sampling of adipose tissue, internal organs and skulls from more than 100 free-ranging polar bears killed by local subsistence hunters from Central East Greenland (69˚00'N to 74˚00'N) during 1999-2002. The present thesis exposes the first and most important results from this large multidisciplinary study of this material, and evaluates the possible connection between the relatively high levels of organohalogens in the adipose tissue and pathological changes in skulls and internal organs. Our results suggested a decrease in adipose tissue concentrations of organohalogens in East Greenland polar bears from 1990 to 1999-2001. Two of the biological effect parameters (FA and enlarged clitoris) did not indicate a link to the relatively high levels of organohalogens. But, there was indications of strong relationships between various organohalogen compounds and skull mineral density indicating disruption of the bone mineral composition. The histopathological changes found in liver- and kidney tissue were a result of ageing, infectious agents, season and meaby chronic exposure to organohalogens. These result fill out an existing knowledge gap in potential effects of environmental, organic contaminants on fluctuating asymmetry, bone mineral density and functional anatomy (histology) in the polar bear. In addition, the results may have a large social importance for Inuits as well.



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# Contents

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# Summary

Marine mammals in the Arctic – and in particular the polar bear (*Ursus maritimus*) in East Greenland – have accumulated considerable amounts of anthropogenic persistent organic industrial chemicals and pesticides (*e.g.* PCBs and DDTs) since *ca.* 1960 (where many of the substances were taken into use) due to these compounds' chemical properties. The pollutants' molecular structures are similar to those of the hormonal steroids / peptides which makes them prone to act as endocrine disruptors of physiologic homeostasis with potential negative biological effects on hormone systems, reproduction and immuno functions. At Svalbard, studies of these effects have been carried out the past 10 years, but still there is a substantial lack of knowledge in this field. Norwegian and Canadian research groups have investigated the toxic effects of these man-made pollutants on external female sexual organs, sex hormones (steroids), cortisol, retinol, thyroid hormones and immunological, reproductive and survival parameters in the Svalbard polar bear population. They found relations between especially PCBs and immunosuppression and alterations in hormone levels, but to date it cannot be concluded whether these are true cause-effect relations (long-term controlled studies on relevant Arctic top predators such as Arctic fox, *Alopex lagopus*, and sledge dog, *Canis familiaris*, are required to fully understand this).

To investigate the relation between biological parameters, not earlier investigated in the polar bear, and organohalogen pollution in East Greenland polar bears, we initiated a sampling of adipose tissue, internal organs and skulls from more than 100 free-ranging polar bears killed by local subsistence hunters from Central East Greenland (69˚00'N to 74˚00'N) during 1999-2002. The present thesis exposes the first, and most important, results from this large multidisciplinary study of this material, and evaluates the possible connection between the relatively high levels of organohalogens in the adipose tissue and pathological changes in skulls and internal organs. These result fill out an existing knowledge gap in potential effects of environmental, organic contaminants on fluctuating asymmetry, bone mineral density and functional anatomy (histology) in the polar bear. In addition, these results may have a large social importance for Inuits as well.

*The first paper* reports the results from the analysis of organochlorines (PCBs, DDTs, CHLs, dieldrin, HCHs and HCB; PBDEs are reported elsewhere) in 92 individual polar bears collected 1999 to 2001. The concentrations showed age and sex differences for all contaminants and seasonal (yearly) patterns for most age and sex groups. The results were compared with samples from 1990 from the same region and this suggested a temporal trend (decline) between ca. 20% and 70% depending on contaminant group. However, final conclusions should not be drawn here as this trend is based on only two different sampling periods within the 10-year period (time trend studies require more than two sampling years). We estimated the half-lives of organchlorine compounds in subcutaneous adipose tissue in the subpopulation of East Greenland polar bears based on the present sample analyses from the two collection periods (*i.e.* 1990 vs. 1999-2001) and over this period the half-lives were in the range of *ca.* 4 to 20 years. In general, the contaminant concentrations were within the range of supposed biological threshold levels of hormone and vitamin concentrations, litter size, reproduction a.o. for mammal wildlife populations and studies in the laboratory.

*The second paper* deals with a time trend in developmental instability (measured as fluctuating asymmetry; FA) in 283 East Greenland polar bear skulls sampled from 1892 to 2002. FA was analysed in relation to 1) period differences (before and after the supposed onset of pollution which is *ca.* 1960) and in relation to 2) individual levels of the analysed organohalogens. Two different analysis showed that for ten bilateral traits the degree of FA did not differ statistically between the two periods, and in four traits FA was higher in the period of no pollution. The analysis also indicated a higher developmental instability in adults compared to subadults (no obvious sex difference was found). A correlation analysis of FA in the skull versus individual levels of the organohalogens in 94 individuals showed no significant trend. The result is possibly influenced by genetic (metabolic), environmental (*e.g.* temperature) and sampling frequency factors which we could not avoid, and in addition the organohalogen exposure could have been below the biological threshold for FA. Beside this we do not know the exposure at early critical life stages (*in utero* and neonatally).

*The third paper* (two parts) is an analysis of bone mineral density (BMD) of hydroxyapatite in 139 skulls and 52 bacula (penile bones) by X-ray (DXA) scanning. The primary goal was to detect changes in the bone mineral content caused by possible endocrine disruption due to the relatively high levels of organohalogens measured in the adipose tissue. These compounds are known to influence the maintenance of bone mineral content by influencing on relevant hormones (sex steroids, thyroid hormones a.o.). The skull BMD was found to be very well correlated with the femur and vertebrale spine, which are the normal measurement sites in humans. This justified the use of the skull as a marker of the bone status. The investigation showed a clear difference in BMD between subadults of both sexes and adults, increasing in the order subadults<adult females<adult males. In addition the BMD increased with age in subadults but not in adults. There were indications of a decrease in skull BMD in old females (postmenopausal?) but this could not be confirmed due to too few observations in this age/sex group. A time trend analysis (as for FA) was used for exploration, and we found that BMD in skulls sampled in the supposed pre-organohalogen period (1892-1960) was significantly higher compared to the supposed organohalogen pollution period (1961-2002) for both subadults and adult males. In addition, a negative correlation between contaminants and skull BMD was found for PCBs and chlordanes in subadults and dieldrin and DDTs in adult males. In addition to the skull BMD, prevalence of periodontitis within each of the two periods (*i.e.* 1892-1960 and 1961-2002) was compared. No period difference was found within each age/sex group, while periodontitis was found to be highly age-related. In conclusion, the significant time trend analysis, as well as the strong negative correlationship between various organohalogens and BMD, suggest that disruption of bone mineral content may have been caused by exposure to organohalogen compounds but other stressors (*i.e.* nutritional and climatic oscilliations) cannot be ruled out.

*The fourth paper* describes liver histology in 88 East Greenland Polar bears (34 subadults, 29 adult females and 25 adult males). In all individuals, nuclear dislocation from the normal cytoplasmic location (central) in parenchymal cells were found and can probably be ascribed to high vitamin A content in Ito-cells, fat accumulation or organohalogens. Furthermore, mild to moderate mononuclear cellinfiltrations were found portally and as lipid granulomas. In addition, bile duct proliferations, portal fibrosis and fat accumulation in hepatocytes (micro- and macrovesiculary) and Ito-cells was found. Some of these findings were related to age and season. Significant

relations between histological changes and individual levels of contaminants in subcutaneous adipose tissue were found but these were not consistent. In conclusion, the findings were similar to several controlled toxicity studies of PCBs, DDTs and dieldrin. The signs of chronic inflammation in relation to triads in most of the bears could be a natural phenomenon in the East Greenland polar bear, caused by *e.g.* infectious agents, and is not necessarily a result from chronic exposure to toxic substances as organohalogens. However, this could not be elucidated because we did not have access to samples from non (or very low) exposed, free-ranging bears. It could not be concluded whether the high lipid content was a function of lipid hyperphagia, fasting, organohalogen toxicity or a combination of these.

*The fifth paper* reports the histology of kidneys and adrenals in 91 and 43 individuals, respectively. Of these, 38 (42%) exhibited glomerulonephritis and / or -sclerosis. In addition, hyalinisation of tubular basement membrane - accompanied by atrophy and fibrosis – was found in these individuals. One case of tubular cellproliferation at the corticomedullary border was found in a 7-year-old male. Tubular hyalin casts were positively correlated with the degree of glomerular and tubular lesions (protein lost) while accumulations of tubular protein droplets and PAS-positive pigments (*e.g.* bile pigment, melanin, haemoglobin or byproducts from the metabolism of plant material but not lipofuscin or haemosiderin) were found in all individuals. We could not evaluate if these lesions had a clinical impact on the bears. Furthermore, focal mononuclear cellinfiltrations were found in diverging degrees of the cortex and medulla, independent of the glomerular and tubular changes. The glomerular and tubular lesions were the same as those found in highly, environmental polluted subpopulations of marine mammals and fish, laboratory animals exposed to PCBs and estrogens, as well as industrial workers chronically exposed to toxic organic chemicals. In addition, there was a clear age dependency for severe glomerular and tubular lesions, as well as indications of old females having a higher prevalence of moderate and severe changes when compared to old males. However, this could not be evaluated statistically due to a relatively low sample size in these two groups. This difference could indicate that mature female polar bears are more sensitive to extrinsic factor - like organohalogens and infectious agents - due to reproductive related cycles of fasting and laction (discussed in Paper I). No significant relations between histopathological changes and levels of organohalogens were found. No histopathological changes were found in the adrenals. We therefore propose that the renal lesions found in the polar bears were a result of ageing, infectious agents, season and meaby chronic exposure to organohalogens. However, a true cause and effect could not be concluded due to the lack of reference material of not exposed individuals and would require an experimental setup using a relevant phylogenetic Arctic top predator.

*The sixth paper* deals with the female reproductive organs. Earlier field observations from Svalbard from early to mid 1990-ies indicated – among others – a relation between high organohalogen exposure *in utero* and *neonatally*, and clitoral enlargement in two adult female polar bears, and clitoral enlargement and lateral urethral opening in two yearlings, respectively. On 9th July 1999 samples from a 23-year-old female, which exhibited a significantly enlarged clitoris resembling those previously reported in adult female polar bears from Svalbard, was obtained at a Inuit hunt outside Scoresby Sound. A first-time ever histological examination of clitoral enlargement in polar bears revealed intense, chronic, ulcerative and perivascular *clitoriditis* showing that the enlargement was an inflammatory reaction - and

not pseudohermaphroditism - probably caused by licking and biting (resembling acral lick dermatitis in the domestic dog). Except for the clitoral enlargement, all dimensions of the external and internal reproductive organs of this bear were similar to a reference group of 23 normal adult female polar bears from East Greenland collected in 1999-2002. Also, the female bear showed normal genotype, and macroscopic examination of her internal reproductive organs indicated that she was reproductively functional. Concentrations of organohalogens in subcutaneous adipose tissue showed that mean levels were up to 3 times lower than in the reference group animals, and lower than the threshold levels of known exposure to these compounds. But, the relatively low levels of analysed OHCs, in the old female polar bear with enlarged clitoris, were probably a result of gestation and lactation, as up to 70% of the total body burden is transferred transplacentally and via lactation from mother to foetus and cub. Therefore, these present levels cannot give any information about the old females' exposure prenatally (*in utero*) during her embryologically development, in which – if the diagnosis had been pseudohermaphroditism – these reproductive organ abnormalities would have been initiated. In conclusion, the histological examination showed that the enlargement was due to inflammation and not pseudohermaphroditism, why at least some of the previously reported pseudohermaphrodite adult female polar bears from Svalbard may in fact have been misdiagnosed.

In conclusion, our results suggested a decrease in adipose tissue concentrations of organohalogens in East Greenland polar bears from 1990 to 1999-2001. Two of the biological effect parameters (FA and enlarged clitoris) did not indicate a link to the relatively high levels of organohalogens. But, there were indications of strong relationships between various organohalogen compounds and skull mineral density indicating disruption of the bone mineral composition. The histopathological changes found in liver- and renal tissue were a result of ageing, infectious agents, season and meaby chronic exposure to organohalogens.

# Resumé

Betydelige mængder svært nedbrydelige organiske miljøgifte, som for eksempel PCBer og DDTer, er på grund af deres fedtopløselige egenskaber ophobet i arktiske havpattedyr – og i særdeleshed den østgrønlandske isbjørn (*Ursus maritimus*) – siden 1960erne. Mange af disse stoffer efterligner kroppens naturlige steroid- og peptidhormoner, hvorved de - gennem påvirkning af den normale fysiologiske homeostase - kan forstyrre hormonsystemer, forplantningsevne og immunologiske funktioner. Selvom disse biologiske effekter er blevet studeret i isbjørne på Svalbard de sidste 10 år, er der stadig mange uafklarede spørgsmål, og på det seneste er der fra norsk og canadisk side fokuseret på effekstudier af kønshormoner, cortisol, vitamin A og skjoldbruskkirtelhormon samt immunologiske, reproduktionsmæssige og overlevelsesrelaterede parametre. I forbindelse med disse undersøgelser er der meget som peger på, at specielt PCBer virker immunsuppremerende, udover at de også ændrer de normale hormonniveauer. Det er dog umuligt at afgøre, om disse resultater er et udtryk for virkelige årsagssammenhænge. Til at afklare dette, er kontrollerede studier af arktiske toppredatorer som f.eks. polarræv (*Alopex lagopus*) og slædehund (*Canis familiaris*) velegnede. Sådanne studier er sat i gang i Norge og på Grønland.

For at afdække ikke tidligere undersøgte biologiske parametre i isbjørne i relation til organiske miljøgifte, startede vi en indsamling af prøver fra subkutant fedtvæv, indre organer og kranier fra mere end 100 østgrønlandske isbjørne (69˚00'N til 74˚00'N) via lokale fangere bosiddende i Scoresbysund i perioden 1999-2002. Nærværende afhandling afrapporterer de første resultater af studiet som søger at afdække, om de relativt høje niveauer af organohalogener opkoncentreret i fedtvævet har påvirket skeletsystemet (kranier) og indre organer.

*Det første manuskript* behandler analyseresultaterne af organokloriner (PCB, DDT, chlordaner, dieldrin, HCH og HCB) i subkutant fedtvæv fra 92 individer indsamlet fra 1999 til 2001. Generelt udviste koncentrationerne køns- og aldersafhængighed, ligesom der var sæsonmæssige variationer for alle aldersgrupper. Disse nye prøver blev sammenlignet med tidligere analyser af bjørne indsamlet i 1990, hvilket viste et fald på mellem 20% og 70% afhængig af hvilken kontaminantgruppe man kigger på. Dog skal man passe på med endeligt at konkludere hvor stort faldet er, idet beregningerne kun bygger på to indsamlingsår (normalt kræver sådanne beregninger mindst 3 indsamlingsår over en 10-årig periode). Beregningerne viste desuden, at halveringstiderne for organokloriner i subkutant fedtvæv for Østgrønlandske isbjørne baseret på indsamlingerne i 1990 og 1999-2001 var fra 4 til 20 år, og dermed afspejler de svært biologisk nedbrydelige kontaminanter. Sammenlignet med tidligere studier af vilde pattedyr og fund i forbindelse med laboratorieforsøg, svarer niveauerne til, hvad man tidligere har benyttet som effektkoncentrationer for mange af de undersøgte parametre i nærværende afhandling.

*Det andet manuskript* analyserer fluktuerende asymmetri (FA) i 283 isbjørnekranier indsamlet i perioden fra 1892 til 2002. FA udtrykker højre-venstresidig forskel for bilaterale mål, og denne forskel er positivt korreleret til, hvor stresset dyret er. Undersøgelsen koncentrerede sig omkring to formål, nemlig en tidstrend analyse og en analyse mellem FA og individuelle

koncentrationer af organohalogener. To forskellige analyser viste, at der ikke var nogen tidsmæssig forskel i 10 forskellige bilaterale mål, mens der for fire bilaterale mål var en tidsmæssig forskel, idet den formodede ikke-forurenede periode fra 1892 til 1960 udviste den største FA. Herudover viste analyserne også, at FA var højere i kønsmodne dyr sammenlignet med unge dyr. En korrelation mellem FA og individuelle koncentrationer af organohalogener i 94 individer viste ikke nogle klare tendenser. Resultatet var sandsynligvis påvirket af andre faktorer end organohalogener såsom genetisk variation (metabolisme), temperatursvingninger og indsamlingsfrekvenser, som vi ikke var istand til at korrigere for. Det kunne også tænkes, at niveauerne af organohalogener ikke var tilstrækkeligt høje til at overdøve den støj som kom fra førnævnte faktorer, og at kontaminantniveauerne var lavere end den biologiske tærskelværdi for FA. Herudover kender vi ikke eksponeringen *in utero* og neonatalt, som er kendt for at være de kritiske stadier med hensyn til udviklingen af FA

*Det tredje manuskript* (to dele) behandler analyser af mineraltætheden (hydroxyapatit, BMD) i 139 kranier og 52 penisknogler ved hjælp af DXA (røntgen) skanning. Det primære mål var at undersøge om de relativt høje niveauer af organohalogener i isbjørnene havde affødt en ændring i knoglesammensætningen (mineraltætheden) via en hormonforstyrrende virkning af f.eks. kønshormoner. De første analyser viste, at der var en fin sammenhæng mellem BMD i kraniet og i hhv. femur og rygsøjlehvirvler - som er de skeletdele der normalt undersøges på mennesker – og derfor kunne kraniet bruges som et generelt udtryk for skelettets mineraltæthed. Resultaterne viste en klar forskel i BMD mht. alder og køn (knogletætheden steg som følgende: unge hunner<unge hanner<kønsmodne hunner<kønsmodne hanner), og at BMD kun var aldersafhængig hos de unge dyr. Herudover var der en tendens til at BMD faldt i gamle hunner over 13 år, hvilket kunne indikere, at et fænomen, som svarer til postmenopausal osteoporose hos mennesker, måske indtræffer hos isbjørne. En tidstrend analyse af BMD viste, at kranier, indsamlet i den formodede ikke-forurenede periode med hensyn til organohalogener (1892-1960), var signifikant højere sammenlignet med kranier fra den formodede forurenede periode (1961-2002), hvilket galdt både unge og kønsmodne dyr. Samtidig viste det sig, at der var en negativ korrelation mellem kranie-BMD og henholdsvis PCB og chlordaner i unge dyr, og mellem kranie-BMD og henholdsvis DDT og dieldrin i kønsmodne hanner. Herudover blev frekvensen af periodontitis sammenlignet mellem de to perioder (henholdsvis 1892-1960 og 1961-2002), og denne analyse viste, at der ikke var nogen periode- og kønsforskel, men at periodontitis var aldersafhængigt. Vi konkluderer derfor, at de overbevisende periodeforskelle, samt de stærke negative korrelationer mellem forskellige organohalogener og kranie-BMD, kan skyldes eksponering af østgrønlandske isbjørne med organiske miljøgifte (organohalogener), men andre faktorers indflydelse (f.eks. ernæring og klimatiske svingninger) kan ikke udelukkes.

*Det fjerde manuskript* beskriver leverhistologien hos 88 isbjørne (34 unge dyr, 29 kønsmodne hunner og 25 kønsmodne hanner) indsamlet i perioden 1999-2002. I næsten alle dyrene fandt vi tegn på kernedislokation sandsynligvis som følge af det høje vitamin A indhold, lipidakkumulering og/eller organohalogen eksponering. Herudover var der varierende grader af mononukleære celleinfiltrationer (hovedsageligt omkring portalområderne), lipidgranulomer, galdegangsproliferationer, portal fibrosering samt lipidakkumulering dels i hepatocytter (mikro- og makrovesikulært) og dels i Ito-celler. Nogle af disse forandringer var relateret til alder og årstid. Enkelte

signifikante relationer blev fundet mellem grader af histologiske forandringer og individuelle niveauer af kontaminanter i subkutant fedtvæv, men det generelle billede viste ingen forskel. Flere af de histologiske forandringer er sammenlignelige med fund indenfor dyreforsøg med PCB, DDT og dieldrin, men de tegn på kronisk inflammation i de fleste af dyrene vi fandt kan godt være normalt forekommende hos den østgrønlandske isbjørn, og er nødvendigvis ikke relateret til en kronisk eksponering af organohalogener. Herudover kan det ikke konkluderes, om lipidakkumuleringen kan være et resultatet af højt spækindtag, faste, organohalogen toksisitet eller en kombination af disse. Det ville derfor være relevant at sammenligne de østgrønlandske isbjørne med ikke - eller eller meget lavt – eksponerede bjørne for at undersøge om sammenhængen mellem kontaminanter og histologiske forandringer er causal. En sådan undersøgelse kunne også tænkes udført som et kontrolleret effektstudium.

*Det femte manuskript* afrapporterer nyre- og binyrehistologi i henholdsvis 91 og 43 isbjørne fra perioden 1999-2002. 42% af disse udviste glomerulonefritis og/eller -sclerose samt hyalinisering af de tubulære basalmembraner. I et enkelt individ (7-årig hanbjørn) var der tubulære celleproliferationer (hyperplasi) i den corticomedullære overgang. I de fleste bjørne var der tubulær dråbeformig hyalin degeneration (protein) eller pigmentaflejringer (galde pigment, melanin, hæmoglobin eller nedbrydningsprodukter af plantemateriale, men ikke lipofuscin eller hæmosiderin) samt varierende grader af medullære hyalincylindre i lumen. Der var en signifikant sammenhæng mellem graden af nyreskader og medullære hyalincylindre, hvilket tyder på, at proteintabet øges i takt med graden af nyreskader (det kunne ikke konkluderes om dette havde en klinisk effekt på bjørnene). Herudover var der – uafhængigt af køn, alder og nyreskader – forskellige grader af mononucleære lymfohistiocytære celleinfiltrationer. De glomerulære og tubulære forandringer var de samme som er fundet hos forurenede vilde havpattedyr og fisk eksponeret for PCBer og østrogener, samt hos laboratoriedyr og industriarbejdere som kronisk er udsat for organisk kemikalieeksponering. Der var en klar aldersafhængighed i graden af nyreskader, og der var tegn på at gamle hunner udviste tydeligere nyreskader end gamle hanner. Materialet var dog ikke stort nok til at konkludere om dette var tilfældet, men det kunne godt tyde på, at kønsmodne hunner, på grund af fedtmobilisering under drægtighed og opfostring af unger, er mere følsomme for ydre påvirkninger, som f.eks. infektioner og organohalogener, sammenlignet med kønsmodne hanner. Vi fandt ingen sammenhæng mellem graden af nyreskader og individuelle koncentrationer af organohalogener. Ligeledes fandt vi heller ingen histopatologiske forandringer i binyrerne. På baggrund af ovenstående foreslår vi, at de nyreskader som vi har fundet i isbjørnene er en funktion af dyrenes alder, infektioner, sæsonbetinget faste og måske en langtidseksponering for organohalogener. Dette kan dog kun kan afgøres ved en undersøgelse af en ikke- eller laveksponeret kontrolgruppe eller i en kontrolleret forsøg med en phylogenetisk relevant topprædator.

*Det sjette manuskript* handler om de hunlige kønsorganer. Tidligere observationer af kønsmodne hunner (og unger) på Svalbared i 1990erne indikerede, at der kunne være en sammenhæng mellem relativt høje niveauer af organohalogen eksponering henholdsvis *in utero* og *postnatalt*, og forstørret klitoris og placering af urinrørsåbninger. Den 9. juli 1999 blev der skudt en 23-årig isbjørnehun udenfor Scoresbysund med forstørret klitoris. Prøver blev udtaget fra denne bjørn og gjorde det muligt for første gang nogensinde at undersøge en forstørret klitoris og tilhørende kønsorganer (ydre som indre) hos vilde isbjørne udsat for organiske miljøgifte. Den histologiske un-

dersøgelse kunne tydeligt fastslå, at der var ikke var tale om pseudohermafroditisme, men derimod intens kronisk klitorisbetændelse som sandsynligvis er opstået under parring (traume) og senere er blevet forværret som følge af automotilitet (slik og bid). Den forstørrede klitoris – og resten af kønsorganerne samt kranie-BMD - blev sammenlignet med et reference materiale på 23 makroskopisk normale kønsmodne hunbjørne fra Østgrønland indsamlet i perioden 1999-2002. Denne sammenligning viste at hunbjørnen var normal og sandsynligvis drægtig. Herudover viste analyser, at hunbjørnen var genotypisk normal, og at koncentrationerne af organohalogener i subkutant fedtvæv var op til 3 gange lavere sammenlignet med referencegruppen, sandsynligvis som følge af mange drægtigheder og laktationsperioder. Det blev derfor konkluderet, at den forstørrede klitoris var opstået som følge af inflammation (betændelse), og at der dermed ikke var en relation til organohalogen eksponering som først antaget. På baggrund af dette er det tænkeligt, at ihvertfald nogle af de voksne hunbjørne med forstørret klitoris man har fundet på Svalbard, ikke har været pseudohermafroditter.

Således viste vore studier i nærværende afhandling tegn på, at der er sket et fald i koncentrationen af organohalogener i subkutant fedtvæv hos østgrønlandske isbjørne fra 1990 til 1999-2001. To af vores undersøgelsesparametre (FA og forstørret klitoris) viste ikke umiddelbart tegn på relationer til de relativt høje niveauer af organohalogener hos bjørnene. Til gengæld var der tydelige tegn på negative relationer mellem organohalogener og mineraltæthed i skeletsystemet (kranier), mens der i lever- og nyrevæv blev fundet forandringer som kunne relateres til alder, infektiøse agens, årstid samt måske organohalogener.

# Preface

Since *ca.* 1960 signficant amounts of anthropogenic and toxic lipophilic compounds (*e.g.* PCBs, chlordanes and DDTs) have accumulated in polar bears from East Greenland, Svalbard and The Kara Sea after long-range transport from North America and Eurasia. Since 1990 the Arctic Monitoring and Assessment Programme (AMAP) has made an effort to investigate temporal and spatial trends of these contaminants. After the Arctic Assessment Reports from Phase 1 and 2 were published in 1998 and 2004, respectively, political and scientific interest of the potential biological effects of these toxic compounds on the polar bear have increased. The present thesis is the primary result from the investigations of ”Effects of contaminants on the Greenland Sea polar bear” and provides informations on biological parameters not earlier investigated in polar bears.

Roskilde, June 2004,

Christian Sonne,

D. V. M., Research Veterinarian

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# Structure of this thesis

This thesis is composed of an introduction that covers existing knowledge of organohalogens in relation to biological effects in the polar bear. This is followed by a description of levels, temporal and seasonal trends in organohalogens for 92 of the bears investigated in the present thesis and additional samples from 1990 (Paper I). Secondly, time trend analysis of skull fluctuating asymmetry and bone mineral density, which also deals with relations to individual levels of organohalogens, are reviewed (Paper II, III-a,b). The last part of the thesis describes the histology of liver, kidney, adrenals and reproductive organs in relation to individual levels of organohalogens (Paper IV, V, VI). Finally, over all conclusion, assessments, perspectives and recommendations are proposed.

## List of publications and manuscripts included in the thesis

**Paper I**: Dietz, R., F. F. Riget, **C. Sonne**, R. J. Letcher , E. W. Born and D. C. G. Muir (2004): Seasonal and temporal trends in Polychlorinated biphenyls and Organochlorine Pesticides in East Greenland polar bears (Ursus maritimus), 1990-2001. Sci Total Environ 331: 107-124.

**Paper II**: **Sonne, C.**, F. F. Riget, R. Dietz, M. Kirkegaard, E. W. Born, R. J. Letcher and D. C. G. Muir (Submitted): Trends in fluctuating asymmetry in East Greenland polar bears (*Ursus maritimus*) from 1892 to 2002 in relation to organohalogen pollution. Sci Total Environ.

**Paper III-a**: **Sonne, C.**, R. Dietz, E. W. Born, F. F. Riget, M. Kirkegaard, L. Hyldstrup, R. J. Letcher and D. C. G. Muir (Accepted with revision): Is bone mineral composition disrupted by organochlorines in East Greenland polar bears (*Ursus maritimus*)? Environ Hlth Persp.

**Paper III-b**: **Sonne, C.**, R. Dietz, E. W. Born, M. Kirkegaard, F. F. Riget, R. J. Letcher and D. C. G. Muir (In prep.): Periodontitis and tooth wear in East Greenland polar bears (*Ursus maritimus*) during 1892-2002.

**Paper IV**: **Sonne, C.**, R. Dietz, P. S. Leifsson, E. W. Born, M. Kirkegaard, F. F. Riget, R. J. Letcher, D. C. G. Muir and L. Hyldstrup (Submitted): Liver histology of free-ranging polar bears (*Ursus maritimus*) from East Greenland. Toxicol Pathol.

**Paper V**: **Sonne, C.**, R. Dietz, P. S. Leifsson, F. F. Riget, E. W. Born, M. Kirkegaard, R. J. Letcher, D. C. G. Muir and L. Hyldstrup (Submitted): Renal lesions in East Greenland polar bears (*Ursus maritimus*) during 1999-2002. J Wildlife Dis.

**Paper VI**: **Sonne, C.**, P. S. Leifsson, R. Dietz, E. W. Born, R. J. Letcher, M. Kirkegaard, D. C. G. Muir, L. W. Andersen, F. F. Riget and L. Hyldstrup (In press): Enlarged clitoris in wild polar bears (*Ursus maritimus*) can be misdiagnosed as pseudohermaphroditism. Sci Total Environ.

# Abbreviations

| | |
|---|---|
| ∑ | Sum of congeners |
| **Ah** | Aryl hydrocarbon |
| **BMD** | Bone Mineral Density |
| **CHLs** | Chlordanes |
| **CT** | Computed Tomography |
| **CYP** | Cytochrome P-450 isozymes |
| **DDD** | Dichloro Diphenyl Dichloroethane |
| **DDE** | Dichloro Diphenyl Dichloroethylene |
| **DDT** | Dichloro Diphenyl Trichloroethane |
| **DXA** | Dual X-ray Absorptiometry |
| **FA** | Fluctuating Asymmetry |
| **HCB** | HexaChloro Benzene |
| **HCHs** | Hexachloro CycloHexanes |
| **HPA** | Hypothalamic-Pituitary-Adrenal |
| ***n*PCBs** | non-ortho, coplanary PCBs |
| **OCs** | OrganoChlorines |
| **OHCs** | OrganoHalogen Compounds (OCs, PBDEs and PFOS) |
| **PBDEs** | PolyBrominated Diphenyl Ethers |
| **PCBs** | PolyChlorinated Biphenyls |
| **PCDDs** | PolyChlorinated DibenzoDioxin |
| **PCDFs** | PolyChlorinated DibenzoFurans |
| **PFOS** | PerFluoroOctane Sulfonate |
| ***p*QCT** | *p*eripheral Quantitative Computed Tomography |

# Background and aims

Since 1990 the 8 Arctic countries (Denmark, Norway, Sweden, Finland, Iceland, Canada, USA and Russia) joined the Arctic Monitoring and Assessment Programme (AMAP). Through this political and scientific cooperation these countries have made a substantial effort, to document the general levels of contaminants as well as investigating temporal and spatial trends of pollutants in the Arctic. Among the conclusions from the first Arctic Assessment Report (AAR-1), was a recommandation to investigate biological effects of these toxic compounds on top predators including the polar bear. Recently, the second Arctic Assessment Report from phase 2 (AAR-2) was published, which still calls for effect studies on higher trophic levels and the present thesis fullfills some of these knowledge gaps.

In AAR-1 and 2 it was stated that polar bears from the Eastern Atlantic Arctic, including East Greenland, have higher levels of persistent organohalogen contaminants (*e.g.* PCBs and DDTs), than reported in tissues of polar bears from populations elsewhere in the Arctic. The Eastern Atlantic Arctic marine ecosystems receive relatively high air- and seaborne input of organohalogens from sources in lower latitudes compared to other Arctic areas, and these are particularly bioconcentrated and subsequently bioaccumulated in waxy and fatty tissues due to their lipophilic properties. Polar bears carry the highest levels of the Arctic predators because they primarily feed on the blubber of ringed seal (*Phoca hispida*) and bearded seal (*Erignathus barbatus*) to maintain thermoregulation and build up energy storage. The levels found in polar bears from East Greenland, Svalbard and the Kara Sea, are similar to those believed to cause negative effects on reproduction and survival of seals from the Baltic Region.

Therefore, studies of adverse biological effects from these toxic levels were initiated at Svalbard by Norwegians and Canadians to fill out the knowledge gap. At Svalbard polar bears are protected and samples from this region can therefore only be obtained from subcutaneous adipose tissue and blood in connection with tagging programmes (satellite telemetry) involving the use of helicopters. From these samples, studies of the relation between organohalogen compounds (*e.g.* PCBs and DDTs) and retinol (vitamin A), thyroid hormones, sex steroids, cortisol, antibody titres and leucocyte activities have been carried out since early 1990s. These studies can in addition, be linked to the movement and behavioural pattern including denning and reproductive activity.

In East Greenland, app. 50 polar bears are killed annually during the subsistent hunting, and the possibility of obtaining unique samples from internal organs and skulls for new investigations was possible. Therefore, researchers from The National Environmental Research Institute and The Greenland Institute of Natural Resources initiated, with great success, a sampling from these bears through local Inuit hunters in the summer of 1999. In addition to the sampling, an extensive interview investigation was conducted on aboriginal knowledge of pathological changes and biology of the East Greenland polar bear. The aim of the present thesis was to describe the histology and possible histopathological changes in various target organs in app. 100 polar bears sampled during 1999-2002 as well as skull morphology and bone mineral density (BMD) in skulls sampled during 1892-2002 and relate these to organohalogen contaminants analysed in bears collected during 1999-2001. Additional investigations on the collected samples will be conducted in the future.

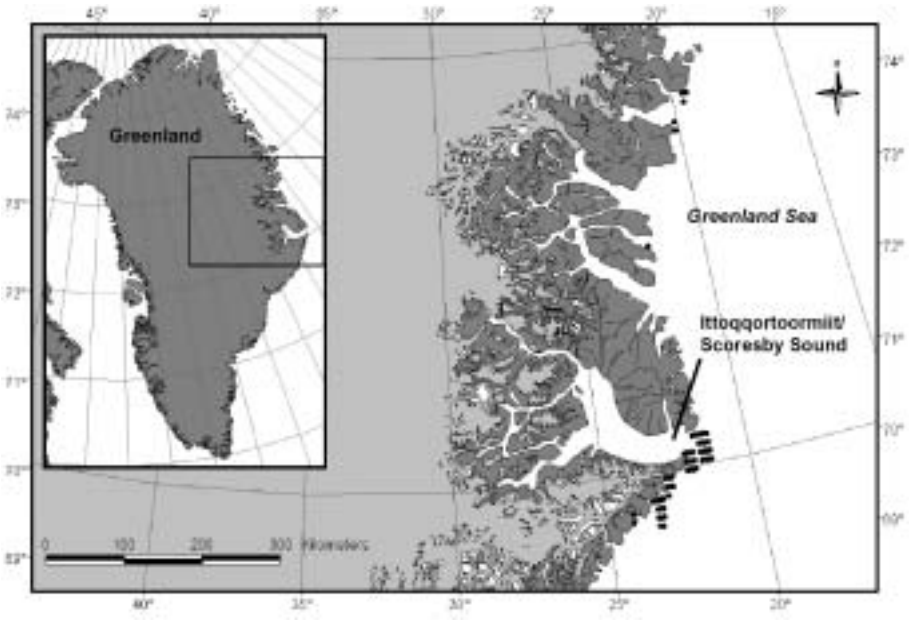
# Introduction

## Study area and sampling method

Samples from more than 100 polar bears were taken between 69˚00'N and 74˚00'N, 19˚00'W and 24˚00'W by local subsistence hunters during 1999-2002 (Fig. 1, 2). It was not possible for scientific personnel to join the hunters along their routes, as these usually have a length of weeks or months during which usually only 1-2 bears are obtained. If scientist based sampling had been chosen, the sampling would have lasted for decades and become extremely expensive. Therefore, hunters were "educated" in taking the samples through local meetings, instruction videos and on a 5-week-long hunt in the spring of 2000, where they were accompanied by a veterinarian. During that hunt the sample quality was assured and sampling problems discussed with the local Inuits.

**Figure 1**
Midnight field sampling by local Inuits at Turner Island, Central East Greenland in April 2000. **Left:** necropsies are taken out. **Right:** Blood samples are preserved (Photo: S. Andersen, Foxtrot).

**Figure 2**
Study area in Central East Greenland 1999-2002. Dots represent samples (adipose tissue, organs and skulls) taken within the municipality of Scoresby Sound.




## Organohalogens

The present investigation focuses on PCBs (polychlorinated biphenyls), DDTs (dichloro diphenyl trichloroethane), CHLs (chlordanes), dieldrin, HCB (hexachlorobenze), HCHs (hexacyclohexanes) and PBDEs (polybrominated diphenyl ethers). Of these, chlordanes, HCHs, Dieldrin and DDTs have been used as chlorinated pesticides and PCBs, HCB and PBDEs as industrial products, in North America and Eurasia for up to *ca.* 60 years, and some of these are still in use (de March *et al.* 1998, AMAP 2004). Depending on their chemical properties and origin of usage, the organohalogens (halogenated aromatic hydrocarbons) are divided into several groups (*Ibid.*). Common for organohalogens is, that one or more hydrogen atoms of the aliphatic or aromatic chain is substituted with a halogen molecule. Chloro-substitution (organochlorines) is the most common while few are brominated (*e.g.* PBDEs) or fluorinated (*e.g.* PFOS) (*Ibid.*). These substances are highly lipophilic and relatively resistant to xenobiotic catabolism and they thereby accumulate in the adipose tissue (*e.g.* seal blubber) of living organisms where they present very long half-lives (*e.g.* Parkinson 1996, de March *et al.* 1998, O'Hara *et al.* 2001, AMAP 2004). In five individual polar bears, studies of *n*PCBs and PCDDs (Polychlorinated dibenzodioxins) and PCDFs (polychlorinated dibenzofurans) (biproducts from the production of organochlorines in high thermal reactions) (*e.g.* de March *et al.* 1998, AMAP 2004) were included in the scientific analysis.

### Bioaccumulation in Arctic animals

The lipophilic organohalogens typically reach the Arctic marine food webs through long distance air- and seaborn transport, from use areas in the mid-latitudes of North America and Eurasia, where they biomagnify in the biota with high lipid content (energy resource) and low metabolism (*e.g.* de March *et al.* 1998, O'Hara *et al.* 2001, AMAP 2004). Subcutaneous adipose tissue is the main lipid storage compartment of the polar bear and the complexity of accumulation and dynamics of organohalogens in these, is related to the dynamics of lipid stores during the cycles of fasting, breeding, lactation, migration etc. (*Ibid.*). In situations where energy is required, lipids and organohalogens are mobilised into the blood stream or are further accumulated depending on the type of contaminant and the type of blubber (lipid

content and composition) varying with location on the body and blubber layer (inner and outer) (*Ibid.*). Bernhoft *et al.* (1997) and Polischuk *et al.* (2002) have shown that up to 70% of the total body burden are transported from mother to offspring via milk during the lactation period. In addition, it is supposed that *in utero* transfer to the foetus is happening as well (e.g. Norstrom *et al.* 1998, O'Hara *et al.* 2001, Polischuk *et al.* 2002, Derocher *et al.* 2003). This poses a great risk for fetuses and *neonatal* individuals and especially the first born cubs offspring is believed to be specially vulnerable to high organohalogen exposure during this stage of life (Norstrom *et al.* 1998, O'Hara *et al.* 2001, Polischuk *et al.* 2002, Derocher *et al.* 2003, Beckmen *et al.* 1999, Ylitalo *et al.* 2001).

### Exposure of East Greenland polar bears

According to Stirling and McEwan (1975) and Kingsley (1998) an "average" polar bear (weight: 200 kg) consumes app. 1000 kg blubber per year. Based on concentrations in ringed seal blubber from East Greenland sampled 1999-2002 ($n$=19) (Riget *et al.* Unpubl. data) the total yearly intake (exposure) or the yearly intake per kg body weight for such an "average" East Greenland polar bear during 1999-2002 was calculated for *4,4'*-DDE ($n$=19; mean=456 ng/g wet weight), *4,4'*-DDT ($n$=19; mean=152 ng/g w.w.), ∑-DDTs ($n$=19; mean=971 ng/g w.w.), dieldrin ($n$=19; mean=87 ng/g w.w.) and ∑-PCBs ($n$=19; mean=1186 ng/g w.w.). These exposure levels are used in the Table comparison across species in the following thesis Chapters (blubber concentrations of organochlorines were not available in lipid weight but as the lipid concentrations was app. 90% on average this was considered sufficient due to the rough estimate of the OC intake). Data on PBDEs in East Greenland ringed seals were not available. Mean concentrations were used in the calculations as earlier studies of contaminant exposure in Inuit Greenlanders have shown this to be the most accurate method (*e.g.* Johansen *et al.* 2000). This is, of course, a rough average estimate of the intake which does not consider exposure time, but it can be used in the comparison with calculated intake or administered doses in other investigations. But still, the overall problem is the extrapolation from species to species and from acute (short-term) to chronic (long-term) toxicity from oral or intraperitoneal exposure. In addition, the calculations reflects exposure and not the actual uptake, as no polar bear toxicokinetic and biotransformation model has been developed yet, and therefore we don't know the bioavailability of these compounds (*i.e.* the amounts entering the portal vein transepithelially depending on molecule size a.o.) nor the toxicodynamic. Beside this, it is also uncertain how much which is metabolised in the liver or peripheral tissue (CYP-isozymes), how much is entering the entero-hepatic circulation, how much is excreted via urine, feces, lungs and milk, and how much is accumulated in periphery adipose tissue. Furthermore, this calculation of exposure cannot take the very important *in utero* exposure during embryologic development into account (*e.g.* Damstra *et al.* 2002) and it cannot estimate the exposure during the 1970s, 1980s and 1990s, in which the organohalogen levels were supposed to be 2-3 times higher compared to today levels (*e.g.* AMAP 2004, Dietz *et al.* 2004).

Data on PCB-126 in ringed seal blubber from 1999-2002 was only available from West Greenland ($n$=5; mean=26.3 pg/g w.w.) (Johansen *et al.* 2004). However, as sum-PCBs have been found to be 2-4 times higher in East Greenland compared to West Greenland, the concentrations were multiplied accordingly (Riget *et al.* In press) which gave a yearly intake of 1.1 µg/kg body weight. No dioxin data on ringed seal blubber from Greenland was yet

available, and hence the intake of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 2378-T4D) in East Greenland polar bears during this period could not be evaluated.

### Metabolism

The mixed function oxidase (MFO) subenzyme cytochromes P-450 isozymes (CYP1A, CYP2B, CYP3A, CYP2E) and epoxide hydrolase located in the smooth endoplasmatic reticulum and microsomes is a ubiquitous group of enzymes in mammals that plays a central role in the oxidative biotransformation (Phase 1; hydroxylation) of organohalogens and other xenobiotics in polar bears and other mammals (*e.g.* Boon *et al.* 1992, Goksøyr and Förlin 1992, Stegeman and Hahn 1994, Goksøyr 1995, Letcher *et al.* 1996, Parkinson 1996, Lewis *et al.* 1998, Lewis 2000, O'Hara *et al.* 2001). When organohalogens are metabolised into even more toxic substances (*e.g.* PCBs to HO-PCBs and $MeSO_2$-PCBs; DDTs to DDE) these are retained in the body through blood-protein binding and/or reaccumulated in adipose tissue (*e.g.* Letcher *et al.* 2000, Guvenius *et al.* 2002) which is also the case for the East Greenland polar bears investigated in the present thesis (*e.g.* Dietz *et al.* 2004, Sandala *et al.* 2004). The microsome Phase 2 conjugation (*e.g.* glucuronidation and sulfation) increases the water solubility and thereby facilitates elimination via renal filtration or entero hepatic excretion (*e.g.* Parkinson 1996). As the CYP-isozymes are upregulated through exposure to organohalogens, this subcellular system has been used as a biomarker for organohalogens in several species (*e.g.* Boon *et al.* 1992, Wong *et al.* 1992, de March *et al.* 1998, O'Hara *et al.* 2001, AMAP 2004) and to date correlation studies of biomarkers in polar bears have mainly been on thyroid hormones ($T_3$/$T_4$), retinol (vitamin A) and sex steroids (*e.g.* Sandau 2001, Skaare *et al.* 2001).

### Toxicity

The physical and chemical properties of organohalogens are determined by the number of halogen atoms and their positioning in the biphenyl ring, and the individual characteristics determine the biological activity of the individual congeners (de March *et al.* 1998). Common for the toxicity of organohalogens are endocrine disruption through their xenobiotic activity in the subcellular enzyme CYP-systems and through their steroid and peptide hormone structures which is similar to several mammal steroid and peptide hormones (*e.g.* de March *et al.* 1998, AMAP 2004). The subcellular organohalogen endocrine disruption is mediated through the cytosolic *Ah*(arylhydrocarbon)-receptor inducing the subsequent gene (DNA) interaction that results in the expression of CYP-related proteins and enzymes (*e.g.* Poland and Knutson 1982, Safe 1984, Safe 1986, Borlakoglu and Haegele 1991, Safe 1991, Colborn *et al.* 1993, Safe 1994, de March *et al.* 1998, Damstra *et al.* 2002, AMAP 2004). The CYP1A and CYP1B type enzymes can bioactivate *endogenous* compounds in the body to toxic forms (*e.g.* 17β-estradiol and estrone into carcinogenic 4-hydroxyestrogens) which may be highly carcinogenic or disrupting the homeostasis of estrogen hormones (*e.g.* van Duursen *et al.* 2003). The CYP1A and CYP1B type enzymes can also bioactivate *exogenous* compounds (anthropogenic) in the body to toxic forms (*e.g.* PCB congener substrates into OH-PCB and other metabolites), which may be endocrine active, carcinogenic or can themselves induce or influence other types of cellular gene expression (*Ibid.*). These properties have the potential of inducing osteoporosis, histopathological changes, immunosuppression, reduced reproductive success, Cushing's syndrome, skin lesions, promotion of carcinogenesis etc.

Of the PCBs found in the East Greenland polar bear, the most biologically active are the dioxin-like *n*PCBs (*non-ortho* PCBs; CBs 37, 77, 81, 126, 169 and 189) which, due to the lack of substitution in the 2nd and 6th position (=*ortho*), allows these to attain a planar configuration similar to the polychlorinated dibenzo-p-dioxins (dioxin-like PCBs). The *mono-ortho* 2,3,3',4,4'-substituted congeners, *e.g.* CBs 118 and 105, are highly toxic as well (*e.g.* Kimbrough 1974, Poland and Knutson 1982, Safe 1984, Safe 1986, Safe 1991, de March *et al.* 1998, AMAP 2004, Sonne *et al.* In press). Another group of highly persistent and highly toxic contaminants found in the East Greenland polar bear are PCDDs (Polychlorinated dibenzodioxins) and PCDFs (polychlorinated dibenzofurans) (Sonne *et al.* In press). Specific in the case of the highly toxic non-ortho and mono-ortho chlorine-substituted PCBs, PCDDs and PCDFs, these have the common characteristic of a potent induction of the aryl hydrocarbon hydroxylase activity via the *Ah*-receptor (cytosolic with high affinity and low capacity that determines the microsomal monooxygenase detoxification enzyme system activity embedded in the smooth endoplasmic reticulum) in hepatic and extrahepatic tissue (*e.g.* Kimbrough 1974, Poland and Knutson 1982, Safe 1984, Safe 1986, Safe 1991, Parkinson *et al.* 1996, de March *et al.* 1998, O'Hara *et al.* 2001, AMAP 2004).

The premise of the TEQ concept applied to non-ortho and mono-ortho chlorine-substituted PCBs is to equate the "dioxin-like" induction capacity via the aryl hydrocarbon receptor (AhR)-mediated mechanism using "mammalian" toxic equivalence factors (TEFs), and thus expressing the $\sum$-coplanart PCBs concentrations after adjustment for the dioxin-like potency relative to 2,3,7,8-TCDD (de March *et al.* 1998, van den Berg *et al.* 1998, AMAP 2004).

Recently, the PCB metabolites (OH-PCBs, and $MeSO_2$) have been considered a great risk to humans as well as marine mammals - including East Greenland polar bears - as these are either retained and/or bioaccumulated in blood-proteins (*e.g.* transthyretin), adipose and/or liver tissue (*e.g.* Haraguchi *et al.* 1992, Bergman *et al.* 1994a, b; Schuur *et al.* 1998a, b; 1999, Sandau 2001, Guvenius *et al.* 2002, Hoekstra *et al.* 2003, Sandala *et al.* 2004).

## Age estimation

The age estimation was done by counting the growth layer groups in the cementum of $I_3$ following Dietz *et al.* (1991) with few modifications (Kirkegaard *et al.* In prep.). To date, this technique has been the only available and relatively precise procedure for estimating the age of free-living mammals. In many of our analysis, the bears were divided into age/sex groups by these criteria: adult males ≥6 years, adult females ≥ 5 years and others as subadults (*e.g.* Rosing-Asvid *et al.* 2002). Therefore, the impact of the relatively low error on the age determination did not influence the results of the statistical analysis in the present thesis.

# Chapter 1
# Levels of organochlorines in East Greenland polar bear subcutaneous adipose tissue from 1990 to 2001

It is of great scientific and social (Inuit) importance to monitor and assess the long-range transport of organohalogen pollution into polar bears. This has been conducted since late 1980s through the international AMAP (Arctic Monitoring and Assessment Programme) circumpolar programme, where research groups from Alaska, Canada, Norway and Denmark have provided samples for analysis of organochlorines (and recently PBDEs and PFOS) at the same two laboratories. A procedure which makes the studies comparable. The first large circumpolar study by Norstrom *et al.* (1998) showed that the highest organochlorine levels in the Arctic were found in polar bears from East Greenland and the European Arctic (Svalbard). Recently, new studies have shown that even higher levels were accumulated in bears from Franz Joseph Land and the Kara Sea of the Western Russia Arctic (Andersen *et al.* 2001, Lie *et al.* 2002). The results in the present chapter is described in details in paper I.

## Levels in East Greenland polar bears 1990-2001

The East Greenland samples were from 1990 and to investigate the levels 10 years after, adipose tissue was sampled from app. 92 polar bears for the present effect study 1999-2001. The adipose tissue was analysed for organohalogens (organochlorines and PBDEs) by gas chromatography with micro electron capture detection (GC-μECD), partly to investigate levels and temporal trends and partly to relate this to possible histopathological changes in target organs (liver, kidney, spleen, thymus, thyroid gland and reproductive organs) and skulls (FA and BMD). The present investigation is the largest to date of organochlorines in a single polar bear subpopulation and provides the first results on a decreasing trend in organochlorines in East Greenland polar bears.

The contaminant groups analysed were persistent organochlorine (OC) contaminants (PCBs, DDTs, chlordanes (CHLs), dieldrin, hexachlorocyclohexanes (HCHs) and chlorobenzenes (CBzs)) sampled from 1999 to 2001 in central East Greenland around Scoresby Sound 69˚00'N and 74˚00'N, 19˚00'W and 24˚00'W). Briefly, the PCBs are the sum (Σ) of the concentrations of the 51 individual or co-eluting congeners (if detected), Σ-DDTs is the sum of 4,4'-DDT, 4,4'-DDD and 4,4'-DDE, Σ-HCHs is the sum of the α-, β- and γ-hexachlorocyclohexane and Σ-CHLs is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. In addition PBDEs (and a subsample of *n*PCBs, PCDDs and PCDFs) were analysed but presented elsewhere (Muir *et al.* In prep., Sonne *et al.* In press) although used in the analysis of biological parameteres in the present thesis (see later Chapters).

### Age, sex and seasonal patterns

The results in Paper I showed, that the concentrations of PCBs and DDTs were significantly higher in East Greenland polar bears compared to Hud-

son Bay bears while the difference was smaller for Σ-CHLs and Σ-CBzs (Norstrom 2001, Fisk *et al.* 2003, Letcher *et al.* Unpubl. data) which is in accordance with earlier reported East / West differences within the Arctic (*e.g.* de March *et al.* 1998, AMAP 2004). In the analysis, individuals were divided into three groups of age and sex: subadults, adult females and adult males. The analysis showed that Σ-PCBs, Σ-CHLs and Σ-DDTs were the dominant classes of OCs in accordance with results from Norstrom *et al.* (1998). Some age / sex differences were found within groups of contaminants (*e.g.* Σ-PCBs, Σ-CBzs, Σ-DDTs, mirex and dieldrin were highest in adult males) which was also the case for seasonal fluctuations (*e.g.* Σ-HCH and Σ-CHL concentrations showed high seasonal variability in adult females) and in agreement with earlier findings (Table 1, Fig. 3) (*e.g.* Polischuk *et al.* 1995, 2002; Bernhoft *et al.* 1997; Norstrom *et al.* 1998). The overall age / sex differences found (males higher than females) were speculated to be a result of both placental transport to the foetus and excretion via milk to cubs during the suckling period (*Ibid.*) or sex specific differences in metabolism (CYP-isozymes) (Norstrom *et al.* 1998, Polischuk *et al.* 2002, Derocher *et al.* 2003). The seasonal variability could partly be explained by depletion of fat deposits (Polischuk *et al.* 1995, 2002).

Due to reproductive cycle periods of fasting, adult female polar bears are suspected to be more sensitive to organohalogen toxicity when compared to adult males. As an example, Arctic seals have as much as 98% of the body burden of contaminants located in the adipose tissue (Stromberg *et al.* 1990) and hence protects the organism from toxic effects on vital organs (Geyer *et al.* 1993, Lassiter and Hallam 1990, Van den Berg *et al.* 1994). Recent studies in free-living harp seals (*Phoca groenlandica*) showed that blood contaminant concentrations could increase more than 7-fold during a period of reduced food intake (Lydersen *et al.* 2002), probably representing a corresponding increased input of the contaminants to sensitive, vital organs. Therefore when polar bear females fast and lactate, relatively high levels of organohalogens are released from adipose tissue to blood and lymph fluids – where they are more or less inactive – to target organs (liver, kidney, adrenals, brain and other high energy and metabolic demanding organs) and thereby become bioavailable and toxic (Polischuk *et al.* 1995, 2002).

When we measure the organohalogen levels in subcutaneous adipose tissue of the East Greenland polar bears, we do not know the exposure in the different life stages (foetus, cub, adult and old) by each individual. Furthermore, we do not know the quantity of absorption, metabolism, mobilisation and excretion which, subsequently, makes the evaluation of potential relations between individual levels of contaminants and our potential effects in biological parameters (FA, BMD and histology) difficult.

**Table 1**
Mean levels± SD of organochlorine concentrations (ng / g l.w.) in East Greenland polar bears sampled during 1999-2001.

| Age/sex group | Σ-PCB | Σ-CBz | Σ-HCH | Σ-DDT | Σ-CHL | Mirex | Dieldrin |
|---|---|---|---|---|---|---|---|
| Subadults (n=50) | 6470±2980 | 158±103 | 198±75 | 462±227 | 2010±1110 | 4.1±6.2 | 218±107 |
| Adult females (n=25) | 8240±5820 | 100±81 | 263±269 | 462±324 | 2220±1540 | 2.8±5.1 | 208±73 |
| Adult males (n=16) | 9100±3560 | 187±252 | 218±67 | 559±441 | 1710±763 | 6.6±11.2 | 245±231 |

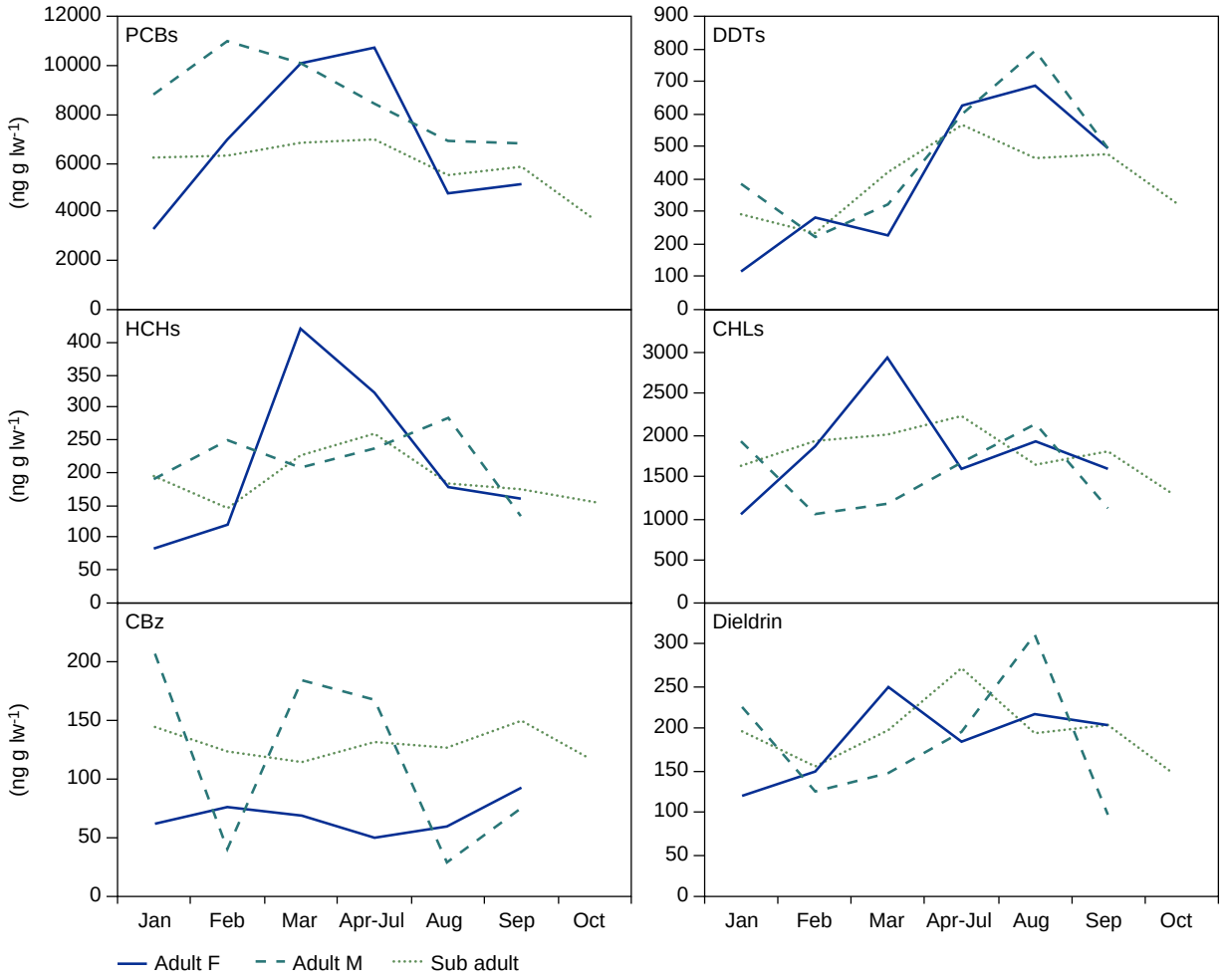



**Figure 3**
Seasonal changes (mean; ng/g l.w.) of PCBs, DDTs, HCHs, Chlordanes (CHLs), benzenes (CBz) and dieldrin for subadults of both sexes, adult females and adult males.

## Time trend

The levels from the 92 bears were compared to samples from 1990 ($n$=17) from the same area reported by Norstrom *et al.* (1998). A time trend analysis showed a significant decline in the levels of organochlorines of *ca.* 28-81% between the 1990 and 1999-2001 bears in both subadult and adult East Greenland bears, indicating half-lives of 4 to 20 years depending on the group of compounds. This was in accordance with analysis of bears from Svalbard (Henriksen *et al.* 2001) and is probably explained by the proximity of East Greenland (and Svalbard) to European sources and the decrease in concentrations of the long-range transport via the air mass movements towards these areas. But no strong conclusions could be drawn about a time trend due to few years of comparisons. Power analysis on polar bears and Greenland ringed seals (*Phoca hispida*) shows that at least 10-12 years are requited to draw such conclusions (Riget *et al.* 2000, Henriksen *et al.* 2001).

Although there are indications of a decrease in the levels of organochlorines in East Greenland polar bears over the period 1990-2001 one must be aware of new contaminants accumulating in the East Greenland polar bears and thereby new potential toxic effects. From the rate of increases in brominated flame retardents in East Greenland polar bears (Muir *et al.* In prep.) it is estimated that these compounds could reach toxicity levels comparable to PCBs within the next 5-10 years if banning will not be enforced. This is also the case of PFOS and related compounds (Smithwick *et al.* In prep., Bossi *et al.* Submitted).

## Individual levels and biological effect parameters

In conclusion, the large individual variability due to sex, age and season makes group comparisons of individual organohalogen levels between degrees of pathological changes within groups of bio-logical effect parameters, difficult (FA, BMD and histology). The levels more or less reflect a "random" subcutaneous adipose concentration due to unknown individual sex (reproductive), age and season history. It would statistically and biologically be easier to compare groups if the body burden levels simply accumulated by age – as *e.g.* mercury in liver and kidney – which then could reflect the total body burden without influence from age, sex and season. Another complexity of the subcutaneous adipose tissue organohalogen content is the unknown biological activity (toxicity) of the levels (more or less inactive when deposited in the adipose but active when relased to the blood stream). The levels of organohalogens and their metabolites in the liver and kidney would perhaps be more useful in the present study. As discussed in the introduction the activity of the metabolites in the bears is unknown although these are known to covariate with the original compound (*e.g.* Sandala *et al.* 2004) and in addition to this is the unknown effect from synergism and antagonism of the compounds.

In the Introduction Chapter we showed that the assumption for calculating a rough estimate of the organohalogen exposure to East Greenland polar bears, is based on the daily intake of known contaminated East Greenland ringed seal blubber. During the period from 1999 to 2002, where the ringed seal data are from, the bears must have been more or less equally exposed per kg body weight (no 10- or 100-fold differences as in rat experiment studies). But of course this calculation does not take into account that the exposure prior to 1999 was significantly higher (which make the old bears relatively more exposed compared to the subadults in our sample) and it does not take into account the mobilisation during gestation and lactation – which probably make adult females more susceptible to organohalogens as discussed earlier. In addition we do not know the *in utero* exposure which may be the most important in studies of organohalogen toxicity. Therefore, when we investigate our effect parameters (FA, BMD and histology) in relation to individual levels of organohalogens in adipose tissue, we compare more or less random levels reflecting age, sex, season, metabolic capacity and individual sensibility to organohalogens at sampling time. This has been pointed out earlier by *e.g.* Pertoldi *et al.* (1997). The optimal way to find cause and effect relation would be the use of a non exposed polar bear subpopulation (or relevant top predator) to compare effect parameters between groups instead of comparing individual East Greenland polar bears more or less equally exposed. This difficulty will be discussed later in relation to FA, BMD and histology.

In the present thesis, the individual levels of organohalogens from the present Paper (∑-PCBs, ∑-DDTs, dieldrin, ∑-HCHs, ∑-CHLs and HCB) as well as ∑-PBDEs, will be evaluated in relation to biological effect parameters (FA, BMD and histopathology) of the individual bears. To our knowledge, this has not previously been conducted on other Arctic mammals.

# Chapter 2
# Fluctuating asymmetry in skulls from East Greenland polar bears collected during 1892-2002

It can be of great importance to monitor the developmental instability in populations of wildlife mammals from a management conservation point of view. Threats to the health status of wildlife today are environmental factors like infective agents (bacteria, virus, parasites), nutrition status (including climatic oscilliations), biotoxins, pollution (heavy metals, organohalogens, noise or other human activities), genetic limitations (bottlenecks) a.o. These stressing factors may affect the fitness of the individual animal and thereby influence the stability of the development of it's "true" phenotype (*e.g.* Palmer and Strobech 1986, Møller 1996, Møller and Swaddle 1997, Rus Hoelzel *et al.* 2002). The developmental instability of the "true" phenotype has been measured in several populations of wild marine mammals (*e.g.* Zakharov and Yablokov 1990, Bergman *et al.* 1992a, Mortensen *et al.* 1992, Schandorff 1997a-b, Coy and Schaeff 2001) through the use of fluctuating asymmetry (FA). FA expresses small (not malformations) "random differences that occur between right and left sides in bilateral traits" (Van Valen 1962, Jagoe and Haines 1985, Palmer and Strobech 1986, Jones 1989, Leary and Allendorf 1989) and earlier investigations have often used the skeletal system (skull) as bilateral phenotype expression due to the relatively easy access to large museum samples of *e.g.* Baltic grey (*Halichoerus grypus*) and ringed seals (*Phoca hispida*) and Danish Kattegat harbour seal (*Phoca vitulina*) (*e.g.* Zakharov and Yablokov 1990, Bergman *et al.* 1992a, Mortensen *et al.* 1992, Schandorff 1997a-b). The access to museum samples have not only the force of relatively easy access to large material, but it also provides the opportunity to investigate time trend analysis in relation to *e.g.* human activities (pollution or hunt) and climatic changes.

In the present investigation it was relatively easy to obtain skull samples (105 pcs.) from the bears from 1999 to 2002 (organohalogen analyses of adipose tissue was only sampled 1999-2001). In addition to these, a large museum skull sample from East Greenland (178 pcs.) was available in Copenhagen, Denmark from 1892-1987. This gave us an opportunity to investigate a time trend in FA in East Greenland polar bears and to relate this to individual levels of organohalogens. The results in the present chapter is described in details in paper II.

## FA in East Greenland polar bears

Fluctuating asymmetry (FA) in 13 bilateral traits (7 in the skull and 6 in the lower jaw) was measured in a total of 283 skulls sampled from 1892 to 2002 (Fig. 4). This was done to investigate a time trend over the entire period and to see if there was a significantly higher FA in the supposed period with organohalogen pollution (1961-2002) compared to the supposed period prior to this pollution (1892-1960). The period 1892-1960 was chosen to represent a period prior to the appearance of organohalogens (PCBs, DDTs, CHLs, dieldrin, HCHs, HCB and PBDEs) originating from long-range transport to East Greenland from southern latitudes. The period 1961-2002 represents the

period where polar bears have been exposed to organohalogens. During this recent period the level of organochlorines is believed to have increased from 1960 to the late 1980-ies followed by a likely decrease from 1990 to 2002.

Within this later period other compounds such as *e.g.* polybrominated flame retardants are believed to have increased throughout the period.

The main result was that no statistical difference between the two periods in 8 of the 13 traits could be detected (Table 2). In five of the traits a difference was found and in these traits FA in skulls from the supposed pre-polluted period of 1892-1960 was higher compared to the supposed polluted period 1961-2002. This was more or less supported by both parametric and non-parametric tests. The time trend analysis (third order polynomial regressions) showed fluctuations in FA over the entire period (1892-2002) in five traits but there was no consistent patterns between these. Regarding age / sex differences, these were found in 7 of the 13 FA-traits (higher in adults compared to subadults). For one trait, adult females were higher compared to adult males (Table 2). The results were similar to those found in harbour seals from Danish waters (*Phoca vitulina*) (Schandorff 1997a-b).

A correlation analysis of FA versus the sum concentrations of various classes of organohalogens in adipose tissue from a subsample of 94 bears from 1999-2001 did not show any significant trends. However, this is not suprising as the concurrent OC concentrations available in the present study do not reflect *in utero* (transgenerational) or neonatal exposure which probably are the most important life stages in the development of organohalogen induced FA (*e.g.* Siegel and Doyle 1975a-c, Doyle *et al.* 1977, Siegel *et al.* 1977a-b, Beckmen *et al.* 1999, Ylitalo *et al.* 2001).

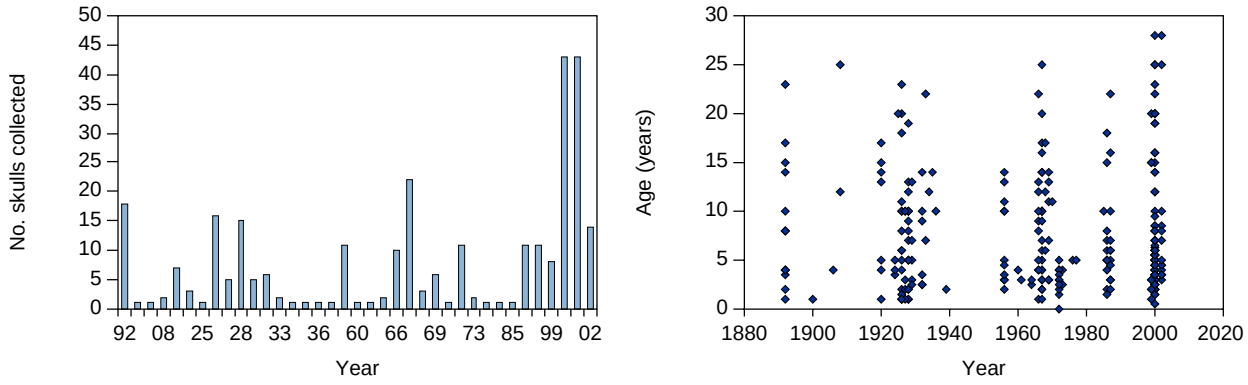



**Figure 4**
**Left:** Number of skulls collected per year from 1892 to 2002 (n=283). **Right:** their individual age.

**Table 2**
Results from the comparison of FA in thirteen traits between periods (1892-1960 vs. 1961-2002) and between age/ sex groups (subadults, adult females and adult males respectively). Note that data from trait 8 were excluded due to high measurement error. Trait 1-7: skull; trait 8-13: lower jaw. n.s.: no significant difference between the periods and age/sex groups, respectively.

| Trait | 1892- 1960 (1) vs. 1960-2002 (2) | Females (F) vs. Males (M) | Females (F) vs. Subadults (S) | Males (M) vs. Subadults (S) |
|---|---|---|---|---|
| Skull | | | | |
| 1 | 1>2 | n.s. | F>S | M>S |
| 2 | 1>2 | n.s. | F>S | M>S |
| 3 | n.s. | n.s. | n.s. | n.s. |
| 4 | n.s. | F>M | n.s. | n.s. |
| 5 | 1>2 | n.s. | F>S | M>S |
| 5 | n.s. | n.s. | n.s. | n.s. |
| 7 | n.s. | n.s. | n.s. | n.s. |
| Lower jaw | | | | |
| 8 | 1>2 | . | . | M>S |
| 9 | n.s. | n.s. | n.s. | n.s. |
| 10 | n.s. | n.s. | n.s. | n.s. |
| 11 | n.s. | n.s. | n.s. | M>S |
| 12 | 1>2 | n.s. | n.s. | n.s. |
| 13 | n.s. | n.s. | n.s. | M>S |

It is seen that for 6 traits (1, 2, 5, 8 and 12) FA was higher in the supposed pre-pollution perid (1892-1960) compared to the supposed pollution period (1961-2002) and that age/sex differencies was found in seven traits (1, 2, 4, 5, 8, 11 and 13).

## FA in mammalian wildlife

Studies of FA in mammalian wildlife in relation to organohalogen pollution, have been conducted the last 15 years (*e.g.* Zakharov and Yablokov 1990, Pertoldi *et al.* 1997, Schandorff 1997a,b). Both the studies of the Danish Kattegat harbour seal (*Phoca vitulina*) population and the Baltic grey seal (*Halichoerus grypus*) population have detected differences in developmental instability over time and correlated the higher FA in skulls to the decades of pollution (*ca.* 1960-recent) (Zakharov and Yablokov 1990, Pertoldi *et al.* 1997, Schandorff 1997a,b).

Of Ursid species, FA has been studied in the Yellowstone grizzly bear (*Ursus arctos*) (Picton *et al.* 1990). In this study FA was associated with genetic limitations (isolation; bottleneck) but not organohalogen pollution. It is not likely that genetic limitations should be higher in the pre-pollution period compared to the pollution period, as a relatively constant hunt has taken place over the last century and no clear change has been observed in the number of bears obtained or the areas where the hunt has taken place (Sandell *et al.* 2001).

## Controlled laboratory studies

Several controlled laboratory studies have correlated FA (dental and bone) to *in utero* disturbances (*e.g.* Siegel and Doyle 1975a-c, Doyle *et al.* 1977, Siegel *et al.* 1977a-b) and therefore it could be speculated that the FA in our East Greenland polar bears could be explained by environmental factors like

temperature extremes and / or food availability (*e.g.* Siegel and Doyle 1975a-c, Doyle *et al.* 1977, Siegel *et al.* 1977a-b, Nilsson 1994, Carrascal *et al.* 1998). Higher climatic fluctuations (against higher temperature) in the first period (*ca.* 1930 and *ca.* 1960) could explain lower food availability and thereby a higher degree of developmental instability in the polar bears compared to the second period (Førland 2002). However, a temperature effect is not likely, as temperatures above normal have been experienced in East Greenland during the last two decades which should limit the food resources (ringed seals) for the bears and thereby increase the FA (*Ibid.*).

## FA and organohalogen levels

No clear pattern in the relationship between FA and individual level of organohalogens was found probably due to the large individual variability in contaminant levels, and because FA likely resulted from prenatal *in utero* disruptions and therefore rather related to contaminant exposure at the time of development than at the time of sampling. Few previous studies of mammals have linked FA to organohalogen contaminant concentrations on an individual by individual basis. Pertoldi *et al.* (1997) examined such correlations (DDTs and PCBs) in the Eurasian otter (*Lutra lutra*), but did not find a relationship between FA and individual contaminant burdens. The authors explained the lack of correlation by the high individual variability of organohalogens including seasonal patterns and sex differences which probably also is the case of the East Greenland polar bear as discussed in Chapter 1. In addition, individual sensitivity may reduce such a pattern especially if the exposure range and number of examined individuals are low.

To give an impression of the levels of Σ-PCBs and Σ-DDTs in the present bears compared to levels in populations of marine mammals where period differences (non polluted and polluted respectively) were found in FA (skulls) and linked to organohalogen exposure, these are compared in Table 3. For Σ-PCBs, the levels in the polar bears were comparable to the lower levels of the Kattegat harbour seal before 1988, where effects on the FA was documented, while for Σ-DDTs the level was 2-10 times lower and it could therefore be suspected that the threshold of FA was not reached in the bears (subeffect exposure) (Blomkvist *et al.* 1992; Schandorff 1997a,b; Zakharov and Yablokov 1990). For the grey seals the differences were even larger. Which compounds cause FA has not been documented to date.

**Table 3**
Range in levels of ∑-PCBs and ∑-DDTs in the blubber (µg/g l.w.) believed to cause FA in juvenile, subadult and adult Kattegat harbour seals (Phoca vitulina) and Baltic grey seals (Halichoerus grypus) populations from before and around 1988 compared to the East Greenland polar bears in the present study. n: number of observations (data from: Blomkvist et al. 1992; Schandorff 1997a,b and Zakharov and Yablokov 1990).

| Study | Compound | n | Concentration | Range in East Greenland polar bear adipose tissue (n) |
|---|---|---|---|---|
| Kattegat harbour seal | ∑-PCBs | 38 | 6-110 | 1-20 (77) |
| Kattegat harbour seal | ∑-DDTs | 38 | 2.0-13 | 0.1-1.1 (77) |
| Baltic grey seal | ∑-PCBs | 37 | 32-5300 | 1-20 (77) |
| Baltic grey seal | ∑-DDTs | 37 | 11.0-1600 | 0.1-1.1 (77) |

In our study we also presented levels of dieldrin, ∑-HCHs, ∑-CHLs, HCB and ∑-PBDEs, but no studies on FA relative to these compounds were available in the litterature.

## Critical comments on the FA concept

It must be mentioned that the FA data exhibited deviation from normality, size dependency and directional asymmetry. We could more or less account for this so we could better meet the necessary assumptions before analysing the data (the directional asymmetry was found to be non-consistent diverging from left to rigth). Based on repeated measurements we managed to correct for measurement error (ME), which has been pointed out to be a large problem in many studies. Some research-ers simply analysed their own measurement error insted of the true FA (*e.g.* Merilä and Björklund 1995).

There is a large uncertainty in measuring FA in wildlife due to the large genetic variability and the accuracy of FA as a measurement for developmental stability is often discussed (*e.g.* Leamy 1992). It seems much better using inbreed strains instead of outbreed stocks (lesser individual biological variability) for FA studies of developmental stability and even when using inbreed strains, large group sizes (in the magnitude of 30 in each group) are often needed to obtain an accurate result (Stub 2003). In addition, when using inbreed stocks, the higher the inbreeding is, the higher is the FA (Leamy 1992).

Swaddle *et al.* (1994) pointed out two potential problems with investigating FA in museum samples: 1) collection of skulls could be biased (in our situation this could mean extraordinary old animals or asymmetric skulls) and 2) it is important to differentiate between “true” FA and FA as a result of wear, damage or malformation. We analysed the length and age difference of the skulls within subadults, adult females and adult males and did not find a significant difference. Therefore we could conclude that the skulls sampled in the period before 1960 was not biased by “trophy” bears (larger, older animals) compared to skulls from individuals collected after 1960. The majority of the skulls collected after 1960 was made up by samples from 1999-2002 ($n$=105), which was considered representative of the Inuit´s catch from that period. This relatively large material was suspected to be the most homogenous of the entire period 1892-2002, and that could result in a lower FA in the pollution period (1961-2002) similar to what we found. Regarding wear, damage or malformations we excluded measurements with ”large” left-right differences (>5mm) from the analysis, so the present investigation should not be influenced by such parameters.

In conclusion, the present study could not document a relationship between skull asymmetry in polar bears and periods with different exposure to organohalogens. These findings are influenced by a numerous number of factors, which we could not control, such as infectious agents (bacteria, virus, parasites), nutrition status (including climatic oscilliations), biotoxins, pollution (heavy metals, organohalogens, noise or other human activities), genetic limitations (bottlenecks and differencies in metabolism) within the two investigated periods. Due to these factors we could not track the individual history of the bears nor the decadal lag from critical exposure phase (*in utero*) to the point of subadult or adult expression (as we have measured). In addition to this we did not have a OHC gradient (dose-response-curve) to examine the changes in FA.

# Chapter 3
# Bone mineral density and periodontitis in East Greenland polar bear skulls collected from 1892 to 2002

Of the 283 skulls analysed for FA, all were analysed for periodontitis and 149 were X-ray scanned for bone mineral density (BMD) to detect signs of osteoporosis. Information on age and gender was unfortunately missing in 10 individuals and therefore only 139 skulls could be used in the further statistical analysis. The purpose was to investigate period differences (1892-1960 vs. 1961-202), a continuous time trend over the entire period (1892-2002) and the relation between individual levels of organohalogens (∑-PCBs, ∑-DDTs, dieldrin, ∑-HCHs, ∑-CHLs, HCB and ∑-PBDEs) in subcutaneous adipose tissue and BMD (BMD was also analysed in 52 bacula in the present investigation). These results are described in details in paper III-a,b.

## Background

Calcium-phosphate (hydroxyapatite) and the double-helix collagen (type 1) are the two major components of bone tissue and determines the hardness and the elasticity, respectively (*e.g.* Ganong 1991, Doige and Weisbrode 1995, Geneser 1996). Bone density expresses the bone mineral content determined by the activity of osteoblastic bone formation and osteoclastic bone resorption which is primarily regulated by parathyroid hormone, androgens and estrogens through cytokines and growth factors (*e.g.* Ganong 1991, Doige and Weisbrode 1995, Manalagas and Jilka 1995, Manalagas *et al.* 1995, Geneser 1996). Exogenous organohalogens have the potential of disrupting this homeostasis through their agonism and antagonism to naturally endogenous hormones (sex steroids, parat hormone, calcitonin, thyroid hormones, cortisol a.o.) leading to dysosteogenesis (osteoporosis, periodontitis a.o.) (*e.g.* Bergman and Olsson 1985, Bergman *et al.* 1992a, Colborn *et al.* 1993, Feldman 1995, de March *et al.* 1998, Sandau *et al.* 2000, Damstra *et al.* 2002, Letcher *et al.* 2002, Hakk and Letcher 2003, AMAP 2004).

Specific in polar bears, recent studies of endocrine disruption on Svalbard of 121 male polar bears have shown that Σ-PCB concentrations (sum of 16 congeners) made significantly contributions to the variation in plasma testosterone levels (Oskam *et al.* 2003). In female polar bears (*n*=86) progesterone was found to be positively correlated with Σ-PCBs (Haave *et al.* 2003) and in both sexes Σ-PCBs and chlorinated pesticides was altering the cortisol levels (Oskam *et al.* 2004). Regarding plasma retinol concentrations and the ratio of total $T_4$ to free $T_4$, these decreased linearly with increasing concentrations of Σ-PCBs in Svalbard bears of varying sex and age investigated in 1991-1994 (Skaare *et al.* 2001). These studies all indicate that organohalogens in Svalbard polar bears (and likely also East Greenland bears, as the OHC levels are comparable) potentially affect the endocrine homeostasis, which again may lead to bone mineral disturbances (osteoporosis, periodontitis a.o.) (*Ibid.*). Another polar bear study from Svalbard have associated high levels of OCs with low levels of IgG suggesting possible immunotoxic effects (Bernhoft *et al.* 2000, Lie *et al.* 2004, Lie *et al.* Submitted). This potential effect may decrease the immune response and enhance stress-induced bone mineral changes through an activation of the hypophyseal-adrenal / thyroid axis, leading to enhanced parathy-

roid and cortisol hormone secretion which subsequently increases bone resorption and decreases bone formation (Selye 1973, Ganong 1991, Colborn *et al.* 1993, Feldman 1995, Damstra *et al.* 2002).

## The method

Our earlier investigations of ringed seals in North West Greenland have shown that the BMD - detected by DXA (Dual X-ray Absorptiometry) scanning - in mandibles reflected the bone mineral density (calcium-phosphate status) of the entire skeletal system (Sonne-Hansen *et al.* 2002). To investigate this in polar bears we measured the skull BMD (*n*=13), the femoral BMD (*n*=13) and the BMD in three lumbar vertebrae (*n*=8) in a sub-set of 13 polar bears from the Copenhagen Zoo and East Greenland. The result showed highly significantly correlations between BMD skull and BMD vertebrae and femur, respectively, which justified the use of BMD in skull to reflect the BMD status of the skeletal system although information on body conditions and nutritional stressors, relevant for osteoblastic and -clastic activity, was not available (Fig. 5, 6).

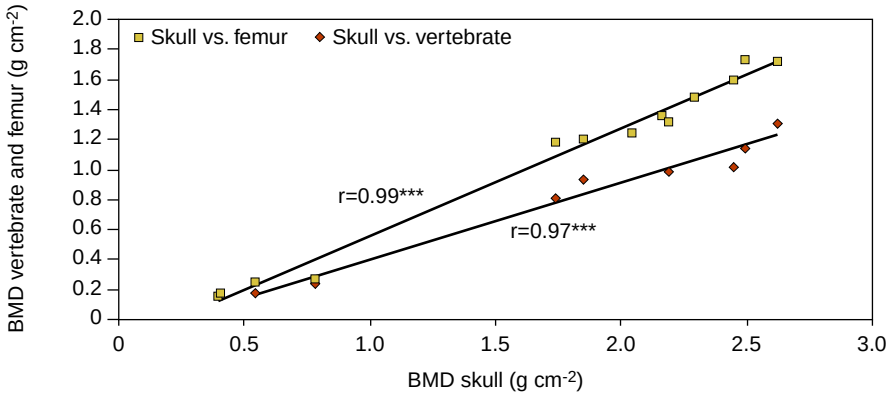



**Figure 5**
Correlation analysis of BMD in skull versus vertebrae (n=8) and BMD in skull versus femur (n=13) in a sub-set of 13 bears from East Greenland and Copenhagen Zoo. Correlation coefficient is given (r).***: indicates p<0.001.

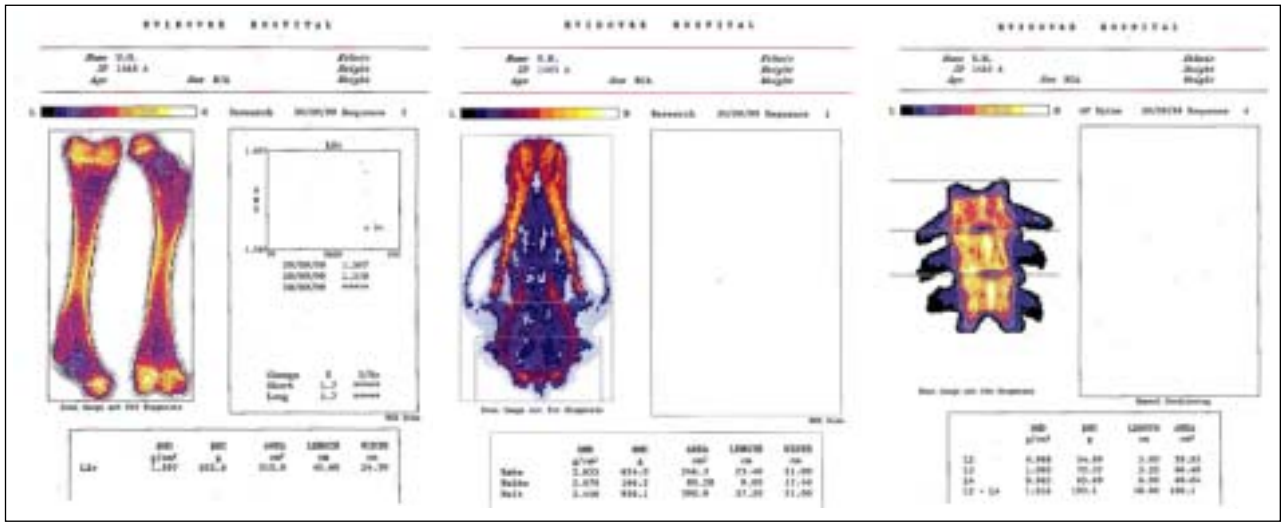


**Figure 6**
**Left:** DXA images from the correlation analysis of femoral BMD (n=13). **Middle:** skull BMD (n=13). **Right:** BMD in three lumbar vertebrae (n=8). The correlation analysis of these are shown in Fig. 5. Note the high density areas of cortical bone tissue (light) and the low density areas of trabecular bone tissue (dark).

BMD analysed by DXA reflects the areal density of hydroxyapatite provided in $g/cm^2$ (Fig. 6). In the clinical evaluation of postmenopausal osteoporosis in *e.g.* the femoral neck or in the lumbal vertebraes the PC-supported images are sufficient to establish the diagnosis of osteoporosis. The DXA BMD expresses the average hydroxyapatite content of both trabecular and cortical bone while CT or *p*QCT have the possibility of expressing the hydroxyapatite in $g/cm^3$ and distinguish between trabecular and cortical bone tissue (*e.g.* Lind *et al.* 2003, 2004). We did not have this possibility but a comparative study between DXA, pQCT and CT is planned as the trabecular and cortical bone tissue may react differently to *e.g.* PCB exposure (*Ibid.*).

## Period differences and time trends

First, we compared 41 skulls sampled 1892-1960 with 98 skulls sampled from 1961-2002. In the preliminary analysis we found that BMD increased by age in subadults, while there was no age dependency in adults of both sexes, and that females showed significantly lower BMD than males. In addition, there were indications of a decrease in skull BMD in old females (postmenopausal) but this could not be confirmed due to too few observations in this age/sex group.

For both subadults and adults, the BMD in skulls from the supposed polluted period (1961-2002) was significantly lower compared to the supposed non-polluted period (1892-1960). This results were supported by the trend analysis over the entire period 1892-2002, and are in accordance with time trend studies of grey seals in the Baltic and the Danish Kattegat harbour seal. In these, the magnitude of alveolar bone loss (osteoporosis) in mandible and maxilla was higher in the supposed pollution period (*ca.* 1960-recent) compared to the supposed prepollution period (before 1960) (Bergman *et al.* 1992a, Mortensen *et al.*1992, Schandorff 1997a). Lind *et al.* (2003) investigated the BMD in mandible and radius of the Baltic grey seal by *p*QCT and found that radius trabecular bone mineral density was significantly higher in a fairly low pollution period (1986-1997) compared to a high pollution period (1965-1985). They also showed that the mandible cortical bone mineral density was significantly lower in the fairly low pollution period (1986-1997) compared to the pre-pollution period (1850-1955).

## BMD and contaminant levels

The second data exploration of BMD was the relationship between organohalogens and BMD on an individual by individual basis. In these analysis we found a negative correlation between ∑-PCBs and ∑-DDTs (and nearly ∑-PBDEs) and BMD in subadults of both sexes while chlordanes and dieldrin were negative correlated to BMD in adult males. In both mammals and birds *4,4'*-DDT is known to be highly estrogenic active through the estrogen receptors (ER), while the metabolites *4,4'*-DDE and also *4,4'*-DDD are less estrogenic (*e.g.* Kupfer and Bulger 1980). At the same time the *4,4'*-DDE metabolite is anti-androgenic – and not estrogenic – through DNA/RNA-transcription blocking effects via ER and/or the androgen receptor (AR) (*e.g.* Kelce *et al.* 1995, Letcher *et al.* 2002). These changes in gene expression are critical for normal biological function (cell proliferation and differentiation) as well as development in the multiple organ systems of mammals. Chlordanes and dieldrin have been shown to have a modulating effect on cytochrome P450 (increase) in rodents, dogs and rhesus monkey and on the

monoxygenase hydroxylation of testosterone (increase) subsequently enhancing the metabolism of these (*e.g.* Zavon and Stemmer 1975, Campbell *et al.* 1983, WHO 1984, Haake *et al.* 1987).

Therefore, it is obvious to believe that the relationship between PCBs, DDTs, chlordanes and dieldrin in adult males and subadults could reflect endocrine disruption against a more estrogenic en-vironment in subadults and adult males (*e.g.* Birnbaum 1994, de March *et al.* 1998, Damstra *et al.* 2002, Lind *et al.* 2003, AMAP 2004, Lind *et al.* 2004). In the case of PBDEs, several studies have correlated these to altered thyroid hormone levels and thereby disruption of bone mineral content (*e.g.* de Wit 2002).

## Osteoporosis in East Greenland polar bears?

Based on above findings of BMD time trends and contaminant relations do we then see indications of osteoporosis? In Table 4 the organohalogen subcutaneous adipose tissue concentrations and exposure (intake) levels in the East Greenland polar bears are compared to levels associated with osteoporosis and dysosteogenesis in wildlife (Bergman *et al.* 1992a, Mortensen *et al.* 1992, Schandorff 1997a, Lind *et al.* 2003, Lind *et al.* 2004), humans (Beard and Jong 2000, Glynn *et al.* 2000, Guo *et al.* 1994, Alveblom *et al.* 2003) and laboratory studies (Lind *et al.* 1999, Lind et *al.* 2000a, b). In one of these environmental studies of humans by Alveblom *et al.* (2003) a relation between organochlorines and increased incidence of osteoporotic fractures in fisherwomen (and -men) was proposed. It is viewed, that the polar bear levels are below or overlapping with exposure levels in environmental studies of wildlife and humans, which indicate that there may be a potential risk for increased incidence of fractures in the bears. It cannot be concluded whether it is osteoporosis, as such demands invasive histological investigations to establish the correct clinical diagnosis and this was not possible. Therefore, our findings indicate that there may be an effect from organohalogen exposure on bone mineral content East Greenland polar bears and that this may increase the risk for fractures.

**Table 4**
Range in the levels (ng/g l.w. or w.w.) of organohalogene compounds associated with osteoporosis and dysosteogenesis in wildlife and laboratory mammals compared to levels measured in the adipose tissue of polar bears in the present study as well as their prey (blubber of ringed seal) and thereby intake. Data from: Bergman et al. 1992[1], Lind et al. 2003[2], Blomkvist et al. 1992[3], Mortensen et al. 1992[4], Schandorff 1997a[5], Lind et al. (2004)[6], Beard and Jong 2000[7], Glynn et al. (2000)[8], Guo et al. (1994)[9], Alveblom et al. (2003)[10], Lind et al. (1999)[11], Riget et al. (In press)[12], Johansen et al. (2004)[13], Lind et al. 2000a, b[14, 15]. ND: below dectection limit.

| Study | Compound | n | Concentration | Concentration in adipose tissue (a) and food (f) of East Greenland polar bears |
|---|---|---|---|---|
| Wildlife: | | | | |
| Grey seal[1, 2] | ∑-DDTs | 38 | 11000-1600000 ng/g l.w. (blubber)[3] | 74-1112 ng/g l.w. (a) |
| Grey seal[1, 2] | ∑-PCBs | 37 | 32000-5300000 ng/g l.w. (blubber)[3] | 898-20407 ng/g l.w. (a) |
| Harbour seal[4, 5] | ∑-DDTs | 38 | 2000-13000 ng/g l.w. (blubber)[3] | 74-1113 ng/g l.w. (a) |
| Harbour seal[4, 5] | ∑-PCBs | 37 | 6000-110000 ng/g l.w. (blubber)[3] | 898-20407 ng/g l.w. (a) |
| Alligator[6] | ∑-DDTs | 16 | ? | 74-1112 ng/g l.w. (a) |
| Humans: | | | | |
| Woman[7] | 4,4'-DDE | 68 | ND-45 ng/g w.w. (serum) | 66-1019 ng/g l.w. (a) |
| Men[8] | Oxychlordane | 115 | 4-36 ng/g w.w. (serum) | 171-6022 ng/g l.w. (a) |
| Men[8] | 4,4'-DDE | 115 | 25-4030 ng/g w.w. (serum) | 66-1019 ng/g l.w. (a) |
| Men[8] | ∑-DDTs | 115 | >25-4140 ng/g w.w. (serum) | 74-1113 ng/g l.w. (a) |
| Men[8] | ∑-PCBs | 115 | >110-1805 ng/g w.w. (serum) | 898-20407 ng/g l.w. (a) |
| Women[9] | ∑-PCBs | 25 | 10 ng/g w.w. (serum) | 898-20407 ng/g l.w. (a) |
| Women (and Men)[10] | ∑-PCBs | 82 | 2000 ng/g w.w. (serum) | 898-20407 ng/g l.w. (a) |
| Laboratory studies: | | | | |
| Rats[11] | PCB-126 | 20 | 7 ng/g body wgt./day (i.p.) | 0.003 ng/g body wgt./day[12, 13] (f) |
| Rats[14] | PCB-126 | 20 | 7 ng/g body wgt./day (i.p.) | 0.003 ng/g body wgt./day[12, 13] (f) |
| Rats[15] | PCB-126 | 20 | 5 ng/g body wgt./day (i.p.) | 0.003 ng/g body wgt./day[12, 13] (f) |

In our study, we cannot prove whether the negative correlation between organohalogens and BMD is true cause-effect relations. This would require a case-control, dose-effect study on polar bears or another Arctic relevant top predator. However, such controlled studies are in progress on domestic Greenland sledge dogs (*Canis familiaris*) and Svalbard Arctic fox (*Alopex lagopus*) in cooperation with Norwegian research groups.

## Macroscopic anatomy and BMD of bacula

Beside the analysis of skull BMD from the period 1892 to 2002, the baculum from a sub-set of 52 East Greenland male polar bears ($n$=52) sampled during 1999-2002 was investigated macroscopically and analysed by DXA-scanning. The statistical analysis applied to the bacula data followed those described in Paper III.

The polar bear penis and bacula were similar to carnivorous in general and all were morphologically normal without any sign of malformation or asymmetry. The BMD in the baculum increased with age, similar to skull BMD, and was significantly positively correlated to BMD in skull ($r$=0.85; $p$<0.001) (Fig. 7). In contrast to the situation in the skulls, BMD in bacula was not correlated with individual levels of contaminants (both: p>0.05; $n$=41). This could probably be due to the lower metabolic activity of cortical bone tissue, compared to trabecular bone tissue, in this specific site of the skeletal system which make it less susceptible to endocrine disruption from organohalogens (*e.g.* Kanis 1997).

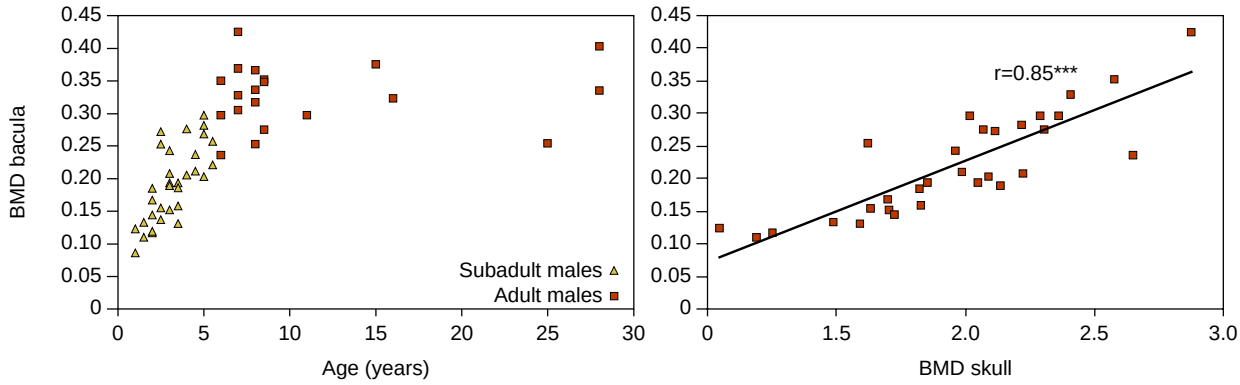



**Figure 7**
**Left:** Bone Mineral Density (g hydroxyapatite/cm$^2$) in 52 bacula of East Greenland polar bears as a function of age. **Right:** Bone Mineral Density in skull. Correlation coefficient is given (r).***: indicates p<0.001.

## Periodontitis

Periodontitis has been linked to organohalogen exposure in wildlife as well as controlled laboratory studies (*e.g.* Bergman *et al.* 1992a, Mortensen *et al.* 1992, Schandorff 1997, Render *et al.* 2000, Render *et al.* 2001). Therefore we compared the prevalence of periodontitis between the two periods. We found an age relation (increase) but no sex nor period difference. In Table 5 the East Greenland polar bear organohalogen subcutaneous adipose tissue concentrations and exposure (intake) levels are compared to studies of wildlife and laboratory mammals and it is seen that the polar bear adipose tissue content of organohalogens, as well as the exposure through prey, is significantly lower compared to five of six cited investigations. The fact that we did not find a difference between the two periods (no pollution and pollution, respectively), could be because periodontitis is not a sufficient sensitive biological indicator of organohalogen exposure in environmental studies.

**Table 5**
Blubber levels of ∑-PCBs and ∑-DDTs (ng/g l.w.) linked to periodontitis in Baltic grey and ringed seals. In addition food level of PCB-126 used in mink studies of periodontitis is shown. For comparison levels in adipose tissue and food of the East Greenland polar bears in the present study is shown at the very right. Data from: Bergman et al. 1992[1], Blomkvist et al. 1992[2], Mortensen et al. 1992[3], Schandorff 1997[4], Render et al. 2000[5]; Render et al. 2001[6], Riget et al. (In press)[7], Johansen et al. (2004)[8].

| Study | Compound | n | Concentration | Concentration in East Greenland polar bear adipose (a) and food (f) |
|---|---|---|---|---|
| Wildlife: | | | | |
| Grey seal[1] | ∑DDTs | 38 | 11000-1600000 ng/g l.w. (blubber)[2] | 73.5-1113 ng/g l.w. (a) |
| Grey seal[1] | ∑-PCBs | 37 | 32000-5300000 ng/g l.w. (blubber)[2] | 898-20400 ng/g l.w. (a) |
| Harbour seal[3,4] | ∑-DDTs | 38 | 2000-13000 ng/g l.w. (blubber)[2] | 73.5-1113 ng/g l.w. (a) |
| Harbour seal[3,4] | ∑-PCBs | 37 | 6000-110000 ng/g l.w. (blubber)[2] | 898-20400 ng/g l.w. (a) |
| Laboratory: | | | | |
| Mink[5,6] | PCB-126 | 20 | 24 ng/g (food) | 0.08 ng/g (f)[7, 8] |

# Chapter 4
# Liver histology of East Greenland polar bears sampled during 1999 to 2002

This investigation was based on liver tissue (and subcutaneous adipose tissue; see Paper I) sampled in East Greenland 1999-2002. Histology samples were fixed in combination of formaldehyde and alcohol (10% of a 35% formaldehyde solution and 90% of a 96% ethanol solution), routinely stained and investigated under light microscope. Samples were from 34 subadults of both sexes, 29 adult females and 25 adult males. Finally it was investigated if there was a relation between presence of histopathological changes and individual levels of organohalogens (∑-PCBs, ∑-DDTs, dieldrin, ∑-HCHs, ∑-CHLs, HCB and ∑-PBDEs) in subcutaneous adipose tissue and skull bone mineral density (BMD). These results are described in details in paper IV.

## Where do we stand?

Several investigations of acute PCB, DDT a.o. liver toxicity have been conducted (*e.g.* Kimbrough *et al.* 1971, Bruckner *et al.* 1974, Jonsson *et al.* 1981, Bergman *et al.* 1992b, Chu *et al.* 1994, MacLachlan and Cullen 1995, Parkinson 1996). These have focused on histopathological changes (LM and TEM) as well as enzymatic activity and few on macroscopic changes (*e.g.* hepatomegaly) while almost none have investigated liver changes in environmental polluted wildlife species (which make our present study difficult). Two exceptions though are PCB polluted cormorants (*Phalacrocorax carbo*) (Fabczak *et al.* 2000) and bream fish (*Abramis brama*) (Koponen *et al.* 2001). The main findings in these studies were fat accumulation, mononuclear cell infiltrations (LM) and proliferation of sER and mithocondria (EM) as well as elevated enzyme activities (microsomal monooxygenase). As these investigations show that liver tissue is highly relevant in PCB toxicity - and as polluted polar bear liver tissue never has been evaluated epidemiologically before - we investigated this in the East Greenland polar bear. The access to samples from here was unique as app. 50 bears are hunted yearly by locals from the same municipality and because we have decades of experience in collaborating with locals (*e.g.* Sandell *et al.* 2001).

Bears shot in this area are almost all healthy (Dietz *et al.* 2001). Therefore we do not know if the samples in our investigation are representative for the health status of the East Greenland population or if we only get samples from healthy individuals (similar to “healthy worker effect”). The opposite problem was the case for the Baltic grey and ringed seal analysis by *e.g.* Bergman and Olsson (1985) and Bergman *et al.* (2001) who collected necropsy samples from stranded and drowned (fishing net) dead seals.

In wildlife, OHCs have also been correlated to histopathology in kidney and thyroid gland in harbor seal (*Phoca vitulina*), grey seal (*Halichoerus grypus*), ringed seal (*Phoca hispida botnica*) and harbour porpoise (*Phocoena phocoena*) (Bergman and Olsson 1985, Schumacher *et al.* 1993, Bergman *et al.* 2001). In Baltic ringed and grey seal, organohalogen contamination has been postulated to result in a syndrome resembling Cushing’s disease (Bergman and Olsson 1985, Bergman 1999) which is discussed in Chapter 6.

## Results and comparisons

The polar bear liver was similar to domestic carnivorous species although interlobular fibrous septa seemed to lack as in brown bear (*Ursus arctos*) (MacLachlan and Cullen 1995, Prunescu *et al.* 2003). Five different tissue changes were found in the light microscopic evaluation of the liver tissue: fat accumulation, mild to moderate mononuclear cell infiltrations, portal fibrosis, bile duct proliferations and nuclear dislocation. The lipid accumulation was thought to be a result of blubber intake (Ramsay and Stirling 1988; Messier *et al.* 1992, Dietz *et al.* 2004), but if organohalogen toxicity (PCBs) plays a role in this morphological appearance cannot be ruled out (*e.g.* Kimbrough *et al.* 1971, Kimbrough *et al.* 1972, Bruckner *et al.* 1974, Bergman *et al.* 1992b, MacLachlan and Cullen 1995, Parkinson 1996). Regarding the lipid granulomas these were thought to be a result of randomly distributed infectious agens in the parenchyma via the portal vein probably ”caught” in the narrow space of Disse (MacLachlan and Cullen 1995). There were no age related differences in the degree of mononuclear cell infiltrations while indications of positive age relation was found for portal fibrosis (Table 6). No such age difference were found for bile duct proliferations, but these were found to be highly related to the degree of portal fibrosis. Overall, there was no significant difference in the prevalence of histopathological changes between adult females and adult males (Table 6).

Seasonal variations was found for hepatocytic lipid content (subadults) and fibrosis (adults) with both being lower in Aug-Oct compared to the rest of the year. Regarding the bile duct proliferation and mononuclear cell accumulations around portal triads, these could be a result of chronic inflammation (liver injury) (MacLachlan and Cullen 1995), and has been described in mink (*Mustela vison*) exposed to PCBs (Bergman *et al.* 1992a) as well as free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) (Rawson *et al.* 1993) and Arctic beluga whales (*Delphinapterus leucas*) (Woshner *et al.* 2002) exposed to mercury. Whether this is due to organohalogens and / or other environmental factors cannot be concluded. In our analysis we found significant differences in mean concentrations of organohalogens between groups of histopathological changes. However, these were not consistent probably due to low sample size. As discussed earlier, high individual variability due to age, sex, season, metabolic capacity and individual sensibility to organohalogens could also play a role. Due to low sample size we could not analyze relations between histopathological changes and individual levels of skull bone mineral density either.

**Table 6**
Results of histological examination of liver tissue from 88 polar bears sampled in East Greenland, 1999-2002 given as frequencies (no. of observations in paranthesis). Changes considered were lipid content of Ito-cells (lipid), mononuclear portal cell infiltrations (infiltrations), granulomes (often lipid granulomes), portal fibrosis (fibrosis) and bile duct proliferation (proliferation). Histopathological findings were classified according to severity (absent, mild and moderate). Sub: subadults of both sexes, AdF: adult females and AdM: adult males. In few cases of autolysis and freeze damage, one or more of the changes considered could not be evaluated.

| Group | Lipid | | | Infiltrations | | | Granulomes | | | Fibrosis | | | Proliferation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sub | AdF | AdM | Sub | AdF | AdM | Sub | AdF | AdM | Sub | AdF | AdM | Sub | AdF | AdM |
| Absent | 41% (14) | 10% (3) | 0% (0) | 3% (1) | 8% (2) | 21% (5) | 16% (5) | 52% (13) | 33% (8) | 55% (17) | 48% (12) | 33% (8) | 68% (21) | 56% (14) | 58% (14) |
| Mild | 26% (9) | 10% (3) | 16% (4) | 66% (21) | 72% (18) | 54% (13) | 68% (21) | 36% (9) | 54% (13) | 45% (14) | 52% (13) | 67% (16) | 32% (10) | 44% (11) | 42% (10) |
| Moderate | 32% (11) | 80% (23) | 84% (21) | 31% (10) | 20% (5) | 25% (6) | 16% (5) | 12% (3) | 13% (3) | . | . | . | . | . | . |
| Total | 34 | 29 | 25 | 32 | 25 | 24 | 31 | 25 | 24 | 31 | 25 | 24 | 31 | 25 | 24 |

**Table 7**
Levels of ∑-PCBs, ∑-DDTs, and PCB-126 linked to liver lesions (mainly fatty liver and mononuclear cell infiltration) in laboratory mammals and wildlife (fish and cormorants). For comparison levels in adipose tissue and food of the East Greenland polar bears in the present study is included at the very right. Data from: Fabczak et al. (2000)[1], Kimbrough et al. (1971)[2], Riget et al. (Unpubl data)[3], Riget et al. (In press)[4], Kimbrough et al. (1972)[5], Bruckner et al. (1974a)[6], Bruckner et al. (1974b)[7], Jonsson et al. (1981)[8], Chu et al. (1994)[9], Johansen et al. (2004)[10], Bergman et al. (1992)[11] and Grinwis et al. (2001)[12].

| Study | Compound | n | Concentration | Concentration in adipose tissue and food of polar bears in the present study |
|---|---|---|---|---|
| Wildlife: | | | | |
| Cormorants[1] | ∑-PCBs | 48 | 0→ > 5 µg/g w.w. (liver) | 0.9-20 µg/g l.w. (adipose) |
| Laboratory: | | | | |
| Rat[2] | Dieldrin | 45 | 0-5 µg/g body wgt./day (food) | 0.001 µg/g body wgt./day (food)[3,4] |
| Rat[2] | DDT | 50 | 0-27 µg/g body wgt./day (food) | 0.01 µg/g body wgt./day (food)[3,4] |
| Rat[5] | ∑-PCBs | ? | 0-72 µg/g body wgt./day (food) | 0.02 µg/g body wgt./day (food)[3,4] |
| Rat[6] | ∑-PCBs | ? | 0-25 µg/g (food) | 1.2 µg/g (food)[3,4] |
| Rat[7] | ∑-PCBs | ? | 0-33mg/kg body wgt./day (i.p.) | 0.02 mg/kg body wgt./day (food)[3,4] |
| Rat[8] | PCB, DDT | 24 | 75-150 µg/g (food) | 1.1-1.2 µg/g (food)[3,4] |
| Rat[9] | PCB-126 | 120 | 0.0001-0.1 µg/g (food) | 0.00008 µg/g (food)[3,4,10] |
| Mink[11] | ∑-PCBs | 70 | 2 mg/day (food) | 3 mg/day (food)[3,4] |
| Flounder[12] | PCB-126 | ? | 0-3 µg/ body wgt./day (food) | 0.000001 µg/ body wgt./day (food)[3,4,10] |

In Table 7 the adipose concentration of the polar bears is compared to laboratory investigations of acute toxicity and wildlife concentrations of dieldrin, DDT, PCBs and PCB-126.

In addition to the adipose tissue concentration, the food concentration and intake was calculated for the comparisons in Table 7 (see Introduction Chapter). It is shown that the concentrations used in the acute toxicity studies were significantly larger compared to the chronic sublethal exposure of the polar bear through their entire lifespan. But on the other side, the polar bears are exposed to a broad mixture of organohalogens. Although the levels differ, there are indications of similar histological changes in these studies when compared to the bears. The changes we found in the polar bear liver tissue were not specific and therefore it was not possible to conclude on the etiology of these, although organohalogens (or mercury) may play a role. To make a final conclusion, a controlled study on a relevant top predator would be preferable as earlier discussed. As a final remark we also investigated the histology of lymph nodes, spleen, thyroid gland and thymus in relation to organohalogens which is reported elsewhere (Kirkegaard *et al.* Submitted).

# Chapter 5
# Histology of East Greenland renal tissue and adrenals sampled during 1999-2002

Kidney and adrenal investigations were based on tissue samples from 91 and 43 bears, respectively, killed in Inuit hunts in East Greenland during 1999-2002 (the histological examination followed the one for liver tissue, Chapter 4). In the evaluation of toxicity and histopathological changes, these were analysed in relation to individual levels of organohalogens in adipose tissue (∑-PCBs, ∑-DDTs, ∑-CHLs, dieldrin, ∑-HCHs, HCB and ∑-PBDEs). Damage to proximal kidney tubules, as well as Cushing's syndrome, can induce demineralisation of the skeletal system (Fanconi's syndrome) leading to osteoporosis (Friberg 1986, WHO 1992). Therefore bone mineral density was analysed in a sub-set of 56 bears in relation to renal histopathological changes. These results are described in details in paper V.

## Renal lesions

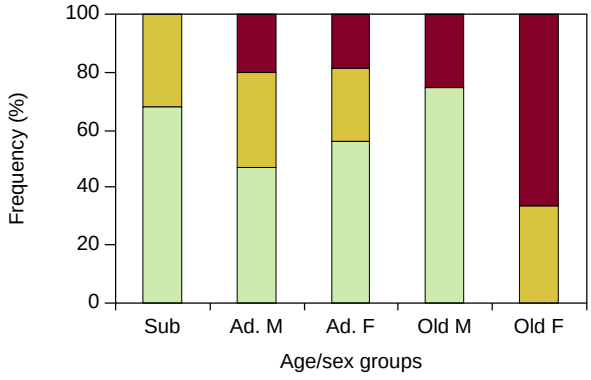
Of the 91 individuals examined, 38 (42%) exhibited glomerular basement membrane thickening (glomerulonephritis and -sclerosis) and different degrees of mesangial deposits. In most of these, hyalinisation of tubular basement membrane (accompanied by atrophy and fibrosis) and glomerular sclerosis were often present while tubular protein droplet accumulations and PAS-positive pigments (e.g. bile pigment, melanin, haemoglobin or byproducts from the metabolism of plant material but not lipofuscin or haemosiderin) were found in nearly all individuals. In one 7-year-old male tubular cell proliferation at the corticomedullary border was found as well. There was a clear age dependency for severe glomerular and tubular lesions, and indications of old females having a higher prevalence of moderate and severe changes when compared to old males (Fig. 8). However, this could not be evaluated statistically due to a relatively low sample size in these two groups.

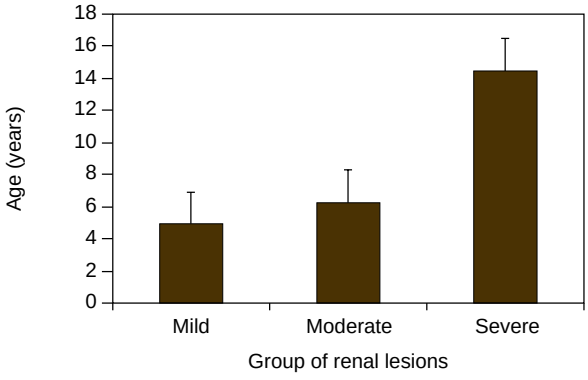
The renal histological changes found in the glomeruli in the present study were similar to those induced by organohalogens – and heavy metals - in controlled laboratory experiments and humans (*e.g.* McCormack *et al.* 1978, WHO 1992, Maxie 1993, Rao *et al.* 1993, Churg *et al.* 1995, Confer and Panciera 1995, Cotran *et al.* 1999, Wade *et al.* 2002). The histopathological changes found in the glomeruli and mesangium of the bears were also to some degree similar to those found in Baltic grey seals and ringed seals exposed to high concentrations of organohalogens during the period 1977-1996 (Bergman and Olsson 1985, Bergman *et al.* 2001) as well as in as free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) (Rawson *et al.* 1993) and Arctic beluga whales (*Delphinapterus leucas*) (Woshner *et al.* 2002) exposed to mercury. Based on a reference material from zoological gardens and Svalbard (non and low polluted, respectively), as well as a large time trend study over the entire period 1977-1996, Bergman and Olsson (1985) revealed a plausible effect of age and chronic exposure to PCBs and DDTs on kidney histology (the relations to individual levels of organohalogens was investigated as well). The same age relations were found in the present study, and based on time trend studies in East Greenland (*e.g.* AMAP 2004, Dietz *et al.* 2004), the renal lesions in the polar bears may be explained by age and

organohalogens as well. Furthermore, the indications of the prevalence of moderate and severe changes being higher in old females - when compared to old males (Fig. 8) - could indicate that adult female polar bears are more systemic exposed to organohalogens due to adipose mobilization and storage dynamics associated with reproductive cycle periods of fasting and lactation (*e.g.* Polischuk *et al.* 1995, 2002).

Sonne-Hansen *et al.* (2002) investigated renal lesions in North West Greenland ringed seals in relation to cadmium toxicity. These seals were later analysed for ∑-PCBs and ∑-DDTs (Table 8) and the histopathological changes found in that study resembled some of those observed in the present East Greenland polar bears. In Table 8 concentrations of organohalogen compounds related to renal lesions in additional studies are listed and compared to the subcutaneous adipose tissue concentration and exposure (intake) of organohalogens East Greenland polar bears of the present studies. It is seen that for each of the contaminant groups, solely, the concentration and exposure of the East Greenland polar bears is lower compared to the studies linking organohalogen compound exposure to renal lesions. However, the bears have been long-term exposed for their entire lives including *in utero* and neonatally in which the susceptibility to lesions is high.

In the renal tissue of the polar bear we found tubular protein droplets and pigments which probably were related to the feed composition (*e.g.* plant material) or season (fasting or hibernation) and / or a result of glomerular or tubular lesions due to *e.g.* age, infections and maybe PCB exposure (Bruckner *et al.* 1974a, b; Confer and Panciera 1995, MacLachlan and Cullen 1995, Bergman *et al.* 2001). In the Baltic seals, hyperplasia of tubular epithelium was found and related to estrogenic exposure of PCBs and DDTs (Bergman *et al.* 2001). Compared to the Baltic grey and ringed seals the levels in the present polar bears were significantly lower, which could explain that we did not find such severe kidney lesions (Blomkvist *et al.* 1992, Bergman *et al.* 2001) (Table 8). In addition, we did not have non-exposed polar bear reference material while the Baltic seal studies had non-exposed zoo seals and relatively low-exposed seals from Svalbard.




| | Sub | Ad. M | Ad. F | Old M | Old F |
|---|---|---|---|---|---|
| Severe | 0 | 3 | 3 | 1 | 4 |
| Moderate | 16 | 5 | 4 | 0 | 2 |
| Mild | 34 | 7 | 9 | 3 | 0 |

**Figure 8**
**Left:** Prevalence (%) of renal lesions in 91 East Greenland polar bears sampled from 1999 to 2002. The lesions are divided by degree of severity (mild, moderate and severe) and age/sex (subadults, adult females and adult males). No. of observations are given in the table. **Right:** Mean age (years) of the individuals categorised into mild, moderate and severe renal lesions. SD shown at the top. Adult males: ≥ 6 years, adult females ≥ 5 years, old males and females: ≥ 15 years and others as subadults.

**Table 8**
Levels of ∑-PCBs, ∑-DDTs, PCB-126 and PBBs linked to renal lesions (glomerulopathy, tubular cell proliferations and interstitial damage) in wildlife, humans and laboratory mammals (polar bear levels in the present study at the very right). Data from: Bergman et al. 2001[1], Blomkvist et al. 1992[2], Sonne-Hansen et al. (2002)[3], Riget et al. (Unpubl. data)[4], Bruckner et al. (1974b)[5], Riget et al. (In press)[6], Koponen et al. (2001)[7], Grinwis et al. (2001)[8], Johansen et al. (2004)[9], Cormack et al. (1978)[10].

| Study | Compound | n | Concentration | Concentration in adipose tissue (a) and food (f) of polar bears in the present study |
|---|---|---|---|---|
| Wildlife: | | | | |
| Grey seal[1] | ∑-DDTs | 38 | 11-1600 µg/g l.w. (blubber)[1,2] | 0.7-1 µg/g l.w. (a) |
| Grey seal[1] | ∑-PCBs | 37 | 49-5300 µg/g l.w. (blubber)[1,2] | 0.9-20 µg/g l.w. (a) |
| Ringed seal[1] | ∑-DDTs | 17 | 4-820 µg/g l.w. (blubber)[1,2] | 0.7-1 µg/g l.w. (a) |
| Ringed seal[1] | ∑-PCBs | 17 | 8-770 µg/g l.w. (blubber)[1,2] | 0.9-20 µg/g l.w. (a) |
| Ringed seal[3] | ∑-DDTs | 27 | 0.1-3 µg/g w.w. (blubber)[4] | 0.7-1 µg/g l.w. (a) |
| Ringed seal[3] | ∑-PCBs | 27 | 0.1-4 µg/g w.w. (blubber)[4] | 0.9-20 µg/g l.w. (a) |
| Laboratory animals: | | | | |
| Rats[5] | ∑-PCBs | ? | 0-33 mg/kg body wgt./day (i.p.) | 0.02 mg/kg body wgt./day (f)[4,6] |
| Bream; flounder[7] | ∑-PCBs | 50 | ? | 0.9-20 µg/g l.w. (a) |
| Flounder[8] | PCB-126 | ? | 0-3 µg/g b.w./day (food) | 0.000001 µg/g b.w./day (f)[4,6,9] |
| Pups and rats[9] | PBBs | 30 | 100 µg/g (food) | 0.02-0.2 µg/g l.w. (a) |

Occupational health investigations of human chronic exposure to organic solvents (hydrocarbons) in industry workers have showed similar glomerular changes (glomerulonephritis) as we found in the polar bears (Zimmerman *et al.* 1975). Beirne and Brennan (1972) – who also associated organic solvents with chronic nephritis in humans - proposed that the complex was of autoimmune origin due to chronic base membrane injury to kidney and/or lung epithelium. This could theoretically be some of the same mechanisms involved in the aetiology and pathogenesis of the renal lesions in the present East Greenland polar bears.

Investigations of bream fish (*Abramis brama*) and asp (*Aspius aspius*) from PCB-polluted freshwater lakes showed changes in the renal corpuscle in both species (Koponen *et al.* 2001). They associated exposure to PCBs with kidney lesions: dilation of the glomeruli (capillaries), mesangial edema (PAS-positive deposits) and adhesion between the visceral and parietal layer of Bowmann's capsule. In the present study it was hard to evaluate the degree of capillary and mesangial dilatation in polar bears, but PAS-positive deposits in the mesangium/glomerular basemembranes were present. The changes found in the fish resembled, to some degree, what we found in the polar bears (Koponen *et al.* 2001).

Grinwis *et al.* (2001) investigated the effect of PCB-126 on the European flounder (*Platichthys flesus*) on CYP1A induction in laboratory experiments. The fish were exposed to a single dose orally (0, 500, 5000 or 50000 µg/kg respectively) and after 16 days the mesonephros - among other tissue - was investigated immunohistochemically. The results showed that groups exposed to 50000 µg/kg PCB-126 induced immunoreactivity in the epithelium of the proximal tubules, as well as in the stationary/circulating mononuclear cells of the heamatopoietic tissue. In Table 8 the levels are compared to the polar bears (see Introduction), and although the concentrations were significantly higher in the fish compared to the bears, it shows that the metabolism of renal tissue (and mononuclear cells) is affected by the pollutants.

Effects of PBBs (PolyBrominated Biphenyls) (100 ppm in diet) exposure was investigated in pups and rats by McCormack *et al.* (1978). It was unfortunately not possible to compare this laboratory study with the PBB exposure in our polar bears due to lack of PBB data in West and East Greenland ringed seals. Although the firemaster BP-6 was highly brominated compared to the PBDEs analysed in the present East Greenland polar bears, we compared the levels and histological effects in the rat study anyway. The overall histological changes in the rats were shrunken glomeruli and a single case of lymphocyte focus. It was hard to evaluate the degree of shrunken glomeruli in the polar bears, but lymphocyte foci were often present.

As discussed in previous Chapters, the relatively equal exposure of individuals and the high individual variation in organohalogen content made it difficult to evaluate statistical differences among groups. Individual levels of organohalogens were not related to histopathological changes while bone mineral density (see Chapter 3) differed between groups of renal lesions (mild, moderate and severe) in adult males. This result could indicate a relation between renal lesions and bone mineral composition (Fanconi's syndrome) (Friberg *et al.* 1986, WHO 1992) or be a result of low sample size.

In conclusion, the renal lesions were the same as those found in previous studies of organohalogen exposure in the laboratory and wildlife. However, it remained inconclusive whether these changes were a result of organohalogen (or mercury) exposure. Furthermore, it could not be detected whether the changes had a clinical impact on the bears. As a final remark, individuals exhibiting mononuclear cell infiltrates in the kidney also exhibited portal infiltrates in the liver.

## Adrenals

Especially DDTs and its metabolites have shown to cause adrenolytic necrosis of the *zona fasciculata* (*e.g.* Brandt *et al.* 1992). Bergman and Olsson (1985) reported a generalised stress syndrome in Baltic grey and ringed seals during 1977-1996 with adrenocortical hyperplasia (Cushing's syndrome) probably related to organohalogen disruption of the HPA axis.

As Bergman and Olsson (1985) did, we investigated adrenal glands in 43 polar bears from East Greenland from which samples were obtained. We could not find evidence of a general or nodular hyperplasia or necrosis in any of the adrenals examined. The only changes we found were hyperemia probably caused by stress due to the hunt. This result could indicate that adrenal histology is not a sufficiently sensible parameter to detect effects from organohalogens in Arctic mammals.

# Chapter 6
# East Greenland polar bear reproductive organs sampled 1999-2002

For the present investigation, reproductive organs were sampled from both females and males. Due to time constrains, only data on sexual organs from the female polar bears are presented in this thesis. However, the penis was investigated macroscopically before the bacula were prepared for the analysis of bone mineral density (BMD, see Chapter 3). The results from the analyses of female sexual organs are described in details in paper VI.

## Pseudohermaphroditic female polar bears?

During helicopter surveys from 1990 to 1997 at Svalbard, Norwegian researchers found 4 presumed female pseudohermaphrodite polar bears (Wiig *et al.* 1998). The four bears – two adults and two sibling yearlings – exhibited severe clitoral enlargement and a morphological investigation by digital manipulation (palpation) revealed a baculum in the two siblings. No signs of baculum were found in the two adult females, but in the siblings the penis-like clitoris possibly contained a baculum and was additionally placed more caudally than is normal. The urethral opening in these siblings was placed laterally. Due to the protection of bears at Svalbard, the researcher could not investigate the entire reproductive tract and take samples for histology. As mentioned earlier, polar bears in East Greenland and Svalbard are among the most polluted bears in the Arctic, and therefore it was suspected that the clitoral enlargement at Svalbard could be a result of endocrine disruption due to ”hormone-like” organohalogens (*e.g.* PCBs and DDTs) causing pseudohermaphroditism (*e.g.* Benirschke 1981, Polani 1981, Acland 1995, Capen 1995, Feldman 1995, Hunter 1995, Mickelsen and Memon 1995, Wiig *et al.* 1998). A definition of a pseudohermaphrodite is: ”an individual with apparently healthy gonads of only one sex but which also has some traits of the opposite sex" (*Anon.* 1994).

Based on the above finding we focused on the female reproductive tract in the present investigation of East Greenland polar bear genitalia. The 9th of July 1999 we were lucky to obtain samples from a 23-year-old female polar bear, exhibiting an abnormally large clitoris, caught outside Scoresby Sound (Fig. 9). Samples for histology were taken from the reproductive tract for a first time evaluation of clitoral enlargement in polar bears.


**Figure 9**
The reproductive organs prior to fixation from the 23-year-old female polar bear with an enlarged clitoris caught outside Scoresby Sound the 9th of July 1999. E: enlarged clitoris.

## Etiology and pathogenesis

Three hypotheses have been proposed to explain the clitoral enlargement in Svalbard polar bears (etiology):

a) First, 21-hydroxylase (21-CAH) deficiency pseudohermaphrodite complex due to congenital adrenal hyperplasia has been proposed because the phenomenon has been reported in several (app. 17) bears (Wiig *et al.* 1998, Derocher Pers. comm.). The enzyme defects inhibits the aromatisation of androgens to estrogens, which subsequently results in clitoral hyperplasia (male characteristic) due to elveated levels of male steroids (pathogenesis) (*e.g.* Feldman 1995, Valentino *et al.* 2000).

b) Secondly, an androgen-producing tumour was proposed to cause the abnormality by Wiig *et al.* (1998) which could indicate that the females were clinically ill.

c) The last explanation was endocrine disruption due to organohalogen exposure (hormon-like substances; see Introduction Chapter). Pseudoher-

maphroditism in wildlife has been reported in 11 indi-vidual mammals: 4 female black bears (*U. americanus*) and one brown bear (*U. arctos*) from Alberta (Cattet 1988), four male bowhead whales (*Balaena mysticetus*) from Alaska (Tarpley *et al.* 1995, O'Hara et al. 2002) and in 4 presumed female polar bears from Svalbard (Wiig *et al.* 1998). Whether the two sibling cubs and two adult females found at Svalbard exhibited pseudohermaphroditism is discussed in the Regional comparison Section.

## The clitoris

The clitoris, *fossa clitoridis*, vaginal opening, uterine horns, adrenals, liver, kidney, spleen and lymph nodes (axillary and inguinal) were examined histologically. The clitoris showed clearly signs of ulceration and inflammation - which was also supported by the size and colour at sampling time – and characterized as chronic-active ulcerative, proliferative and perivascular clitoriditis. In fact the lesion resembled "acral lick dermatitis" seen in *e.g. Canine* species (Yager and Scott 1993). The initial cause of the lesions could not be determined, but *e.g.* trauma from mating in March-April could have caused the first lesions. Subsequently, licking and biting of the lesions by this individual bear have caused further epithelial damage following persisting inflammatory reaction with formation of granulated tissue. This is supported by the fact that the adult Svalbard female polar bears exhibited signs of inflammation (pus and ulceration) (Ø. Wiig Pers. comm). Therefore, the clitoral enlargement was mainly due to inflammation.

## Reproductive tract

The 23-year-old female polar bear was investigated and compared with a reference material consisting of 4-23 adult female polar bears (≥5 years) sampled in East Greenland during 1999-2001. The vulva (*labia minores* and *majores*) were normal in location and size (though the size could indicate that she was in postestrous). The uterus was morphologically normal but the size was larger than the reference material which could be due to random biological variation or gestation. Both size of vulva and the possible signs of gestation were in agreement with the mating season for East Greenland polar bears which is believed to take place in April-June (*e.g.* Rosing-Asvid *et al.* 2002).

Based on the above findings it seemed that the bear was reproductively healthy. In addition the ovaries had a normal number of follicles in both ovaries and a *corpus luteum*, indicating that she had mated during spring and perhaps was in gestation. The uterine endometrial histological appearance showed highly developed uterine glands (crypts) when compared to subadults, indicating that this female had been in gestation earlier in her life span. No signs of stenosis or occlusions were found in the uterine tubes (oviducts).

Histological examination of the liver and kidney of this individual showed signs of chronic inflammation as described in Chapter 4 and 5. No histopathological changes were found in adrenals, spleen and lymph nodes. No sign of a demineralisation of the skeletal system were found - based on bone mineral density (BMD) in the 23-year-old female polar bear compared to the reference material – although this could be expected in the case of untreated hyperadrenocorticism (21-hydroxylase deficiency) where clitoral megaly is a

common sign (Acland 1995, Feldman 1995, Mickelsen and Memon 1995, Valentino *et al.* 2000). In addition we found that the 23-year-old polar bear genetically was a female based on absence of the SRY / ZFY bands and presence of ZFX band (Wiig *et al.* 1998).

## Levels of organohalogens

The concentrations of ”standard” OHCs in the 23-year-old female polar bear did not differ significantly from the 23 adult reference female polar bears. One exception was ∑-PCBs, which was slightly lower. In general the levels were relatively low – probably due to gestation and lactation – and the ∑-PCBs levels were lower compared to known thresholds for biological parameters (*e.g.* reproduction) (Bernhoft *et al.* 1997, de March *et al.* 1998, Polischuk *et al.* 2002, AMAP 2004).

TEQ values of *n*PCBs, PCDDs and PCDFs were calculated for the 23-year-old female polar bear as recent studies have shown that organohalogen induced CYP1B1 and CYP1A1 can disrupt the homeostasis of estrogen hormones (*e.g.* van Duursen *et al.* 2003). The total TEQ concentration comprised of *n*PCBs, PCDDs and PCDFs in the female pseudohermaphrodite was 3.78 pg / g l.w. and compared to de March *et al.* (1998) and AMAP (2002) the total TEQ value was 100-1000 times lower than threshold levels known from seals and mink; and is generally comparable to terrestrial biota low on the food web. As for the ”standard” organohalogens it is likely that the total TEQ value is lower than earlier in her life cycle (Bernhoft *et al.* 1997, de March *et al.* 1998, Polischuk *et al.* 2002, AMAP 2004). In fact, based on time trends in the Arctic (*e.g.* AMAP, 2004, Dietz *et al.*, 2004), the general OC exposure during her embryonic development was significant higher due to her heavily polluted mother, who is suspected to have carried organohalogen loads 2-3 timer higher.

## Regional comparisons

The observations of clitoral enlargement in two adult female polar bears at Svalbard were similar to that of the East Greenland female and at least one of the Svalbard adults showing signs of earlier gestation and birth (milk in mammary glands), indicating that she had recently weaned and maybe lost her offspring. This could link the clitoral enlargement in these adult bears to events associated with trauma-related lesions around mating, initiating a chronic inflammatory responses in the perineal region.

The two Svalbard yearlings could of course not be evaluated for reproductive function and it is unlikely that these have been subjected to mating attempts or other trauma leading to lesion formation. The two cubs also differed morphologically from the Greenland adult in the caudal placement of the penis-like clitoris and the lateral opening of the urethra. Therefore the ‘pseudohermaphroditic cubs’ cannot be linked to the present female with clitoral enlargement due to trauma, and their anomalous organs might be explained by *e.g.* 21-hydroxylase deficiency (21-CAH) congenital pseudohermaphrodite complex as proposed previously by Wiig *et al.* (1998) or be true pseudohermaphrodites (Benirschke 1981, Polani 1981, Acland 1995, Capen 1995, Feldman 1995, Hunter 1995, Mickelsen and Memon 1995).

In conclusion; pseudohermaphroditism is a rare phenomenon in Ursids as only 9 individuals have been reported. Moreover, androgen-producing tumours – but not Freemartinism as more than two cubs is rare - are likely to cause clitoral enlargement (Wiig *et al.* 1998). Meanwhile, the female polar bear did not differ from the reference group in any of the analysed physiological parameters. We therefore propose that clitoral enlargement in the present adult female polar bear is not a result of endocrine disruption linked to persistent OC contaminant exposure, but rather an inflammatory response probably initiated at *e.g.* mating. Hence, at least some of the supposed pseudohermaphroditism in female polar bears reported in the past probably originated from misdiagnoses. Therefore, future studies examining pseudohermaphrodism in wildlife should consider that certain occurrences are natural events.

# Conclusions and final assessments

Our results suggested a decrease in adipose tissue concentrations of organohalogens in East Greenland polar bears from 1990 to 1999-2001. Two of the biological effect parameters (FA and enlarged clitoris) did not indicate a link to the relatively high levels of organohalogens. But there were indications of a strong relationship between various organochlorines and skull mineral composition which could reflect endocrine disruption. In liver- and renal tissue, the histopathological changes were ascribed to age and infectious agents. However, based on knowledge from wild marine mammals, humans and controlled laboratory experiments, long-term exposure to organohalogen compounds and mercury cannot be ruled out as co-factors.

*The organohalogen* levels measured in the subcutaneous adipose tissue from the East Greenland polar bears colleced 1999-2001 did not tell us anything about the exposure in different life stages (foetus, cub, adult and old) by each individual but gave us a level at time of sampling. Furthermore, we do not know the quantity of absorption, metabolism, mobilisation and excretion which, subsequently, makes the evaluation of potential relations between individual levels of contaminants and our potential effects in biological parameters (FA, BMD and histopathology) difficult. In addition, the large variability in the OC concentrations of adult females showed that OC lipid mobilization from peripheral tissue (primary fat) during reproductive cycles and associated conditions occurs and thereby impact on the results (*e.g.* Polischuk *et al.* 1995, 2002).

Beside the uncertainty of the organohalogen concentrations to reflect the exposure during critical stages of life the relation between organohalogen concentrations and our biological effect parameters studied (FA, BMD and histology) is influenced by many factors which we cannot control or correct for. To get an overview, a theoretical model describing the events of interactions between the parameters studied and extrinsic factors affecting the intrinsic feedback-loops is shown in Fig. 10.

As viewed in Fig. 10, many uncontrolled factors influenced the study of fluctuating asymmetry. Therefore we could not track the individual history of the bears nor the decadal lag from critical exposure phase (*in utero*) to the point of subadult or adult expression which we measured. Regarding the relation between individual organohalogen concentrations in subcutaneous adipose tissue and fluctuating asymmetry we did not have a OHC gradient (dose-response-curve) to examine the changes in FA in *e.g.* non, low and high exposed groups. The concurrent OC concentrations available from 1999-2001 do not reflect *in utero* (transgenerational) or neonatal exposure which probably are the most important life stages in the development of organohalogen induced FA (*e.g.* Siegel and Doyle 1975a-c, Doyle *et al.* 1977, Siegel *et al.* 1977a-b, Beckmen *et al.* 1999, Ylitalo *et al.* 2001). It is not possible to say whether the levels found in the present East Greenland polar bears are high enouth to increase skull fluctuating asymmetry in the bears (a dose-response, case-control study is needed for such an investigation). It is not possible to say whether there could be a potential association between FA and the other effect parameters (BMD and histopathology) investigated.

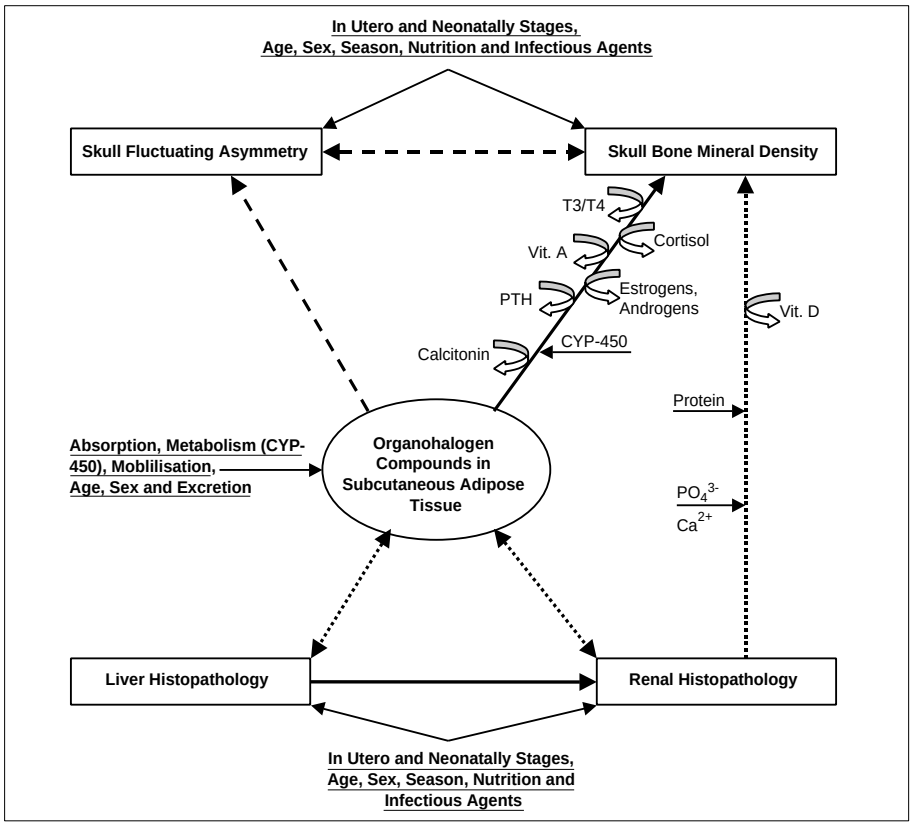



**Figure 10**
The proposed relationships between levels of organohalogen compounds measured and effect parameters studied based on results and hypothesis from the present thesis is given with large bold lines and arrows . Uncontrolled enxtrinsic factors (age, sex, nutritional factors, infectious agents, in utero and neonatally stages) as well as unknown intrinsic factors (absorption, metabolism, mobilisation and excretion) affecting the study is given as underlined text with thin lines and arrows. Proposed involved endocrine feedback loops are shown (lack of OHC gradient [dose-response] is not given as this was a general weekness of the study) with broad curved arrows. In the cases of two-way bold arrows there is a theoretically interaction.

: Statistical significant correlations suggested a link.
: Probabaly a link but we could not prove it.
: No or only tentative links.
: Uncontrolled factors.
: Endocrine feddback loops.

*Bone mineral density* expresses the bone mineral composition determined by the activity of osteoblastic bone formation and osteoclastic bone resorption which is regulated by androgens and estrogens through cytokines (Manalagas and Jilka 1995; Manalagas *et al.* 1995). Studies on Svalbard have shown that PCB may negatively influence plasma testosterone levels (Oskam *et al.* 2003), cortisol levels (Oskam *et al.* 2004) and plasma retinol and thyroid hormone levels in polar bears (Skaare *et al.* 2001) which subsequently may lead to bone mineral loss (osteoporosis) (*Ibid.*).

The endocrine loops implicated in the bone loss could be an enhancement of the hypophyseal-adrenal / thyroid axis, leading to enhanced parathyroid and cortisol hormone secretion which subsequently increases bone resorption and decreases bone formation (Selye 1973, Ganong 1991, Colborn *et al.* 1993, Feldman 1995, Damstra *et al.* 2002) (Fig. 10). We showed that high concentrations of Σ-PCBs and Σ-CHLs were associated with reduced skull BMD in subadults and that Σ-DDTs and dieldrin are associated with reduced skull BMD in adult males. These relationships could be a result of agonistic and antagonistic estrogen receptor-mediated activity, or OC-mediated induction

of CYP-450 activity that impact circulating sex hormone levels (*e.g.* estrogens and androgens) (*e.g* AMAP 2002, Birnbaum 1994, Damstra *et al.* 2002, de March *et al.* 1998, Lind *et al.* 2003, Lind *et al.* 2004, Navas and Segner 1998). Such an endocrine modulation by organohalogen compounds has earlier been demonstrated for polar bears (*Ursus maritimus*) (*e.g.* Letcher *et al.* 1996). The negative correlation between organohalogen compounds (*e.g.* 4,4'-DDE) and BMD have earlier been described in humans (*e.g.* Beard and Jong 2000; Glynn *et al.* 2000).

In conclusion on the skull measurements of FA and BMD, we found a lower bone mineral density (high stress) and a low FA (low stress) in the skulls collected 1961-2002 when compared to 1892-1960 and therefore the two studies do not support each other. The skulls investigated for BMD and FA were the same and environmental factors such as nutrition status, nutrition composition, climate oscilliations and pollution did not differ between the two studies. The different response in FA and BMD parameters over the two parameters could therefore be due to different sensitivity and/or specificity of these two variables to reflect stress.

Both *renal and liver lesions* showed clear relationships to age (increasing) and for renal lesions these were more frequently observed in old females and with higher severity which may be a result of a more systemic exposure to organohalogens due to adipose mobilization and storage dynamics associated with reproductive cycle periods of fasting and lactation (*e.g.* Polischuk *et al.* 1995, 2002). Other aetiological factors like infectious agents and food composition may have played a role as well and we could not conclude whether or not organohalogen pollution or mercury was a co-factor in the development of the lesions. Concentrations used in the acute toxicity studies were significantly larger compared to the chronic sublethal exposure of the polar bear but on the other hand, the polar bears were exposed to a broad mixture of organohalogens which may have increased the risk for organohalogen induced histopathological changes.

As depicted in Fig. 10 the hepatic and renal lesions may have a relation to organohalogens based on the morphology of the histopathological changes. The hepatic lipid accumulation was not comparable to lipidosis while there were several similarities between the renal lesions and glomerulonephritis in humans, laboratory mammals and wildlife. For example chronic nephritis induced by organic solvents in humans have been proposed to be of autoimmune origin due to chronic base membrane injury to kidney and/or lung epithelium. This could theoretically be some of the same mechanisms involved in the aetiology and pathogenesis of the renal lesions in the present East Greenland polar bears.

Furthermore, the indications of a difference in BMD between different degrees of renal lesions could indicate a damage to the proximal kidney due to renal osteodystrophy (osteoporosis) (Friberg *et al.* 1986, WHO, 1992). However, the sample size was low and therefore the result is not reliable but needs a higher sample size in the future. Whether the significant relation between renal and hepatic mononuclear cell infiltrations as well as the similarities with earlier studies of organohalogen induced lesions and the negative correlation between BMD and organohalogens supports a broader theory of organohalogen toxicity, remains unanswered. A controlled case-control, dose-response study is needed to make firm conclusions on these potential relationships

# Perspectives and recommendations

The present thesis contributes significantly to the understanding of the potential toxicity from organohalogen compunds in Arctic marine mammals. It fills out knowledge gaps of effects on bone and tissues necessary for the maintainance of endogeneous homeostasis. It clearly shows that more research in this field is needed to understand the potential effect on a population level for top predators – as the polar bear, Arctic fox and killer whale – in the Arctic.

## Contaminants

Although we have showed that there may be a decrease of contaminants in the East Greenland polar bear from 1990 to 1999/2001, new contaminants are accumulated in the East Greenland marine food webs. New studies on brominated flame retardants (PBDEs) (Muir *et al.* In prep.) and PFOS (Smithwick *et al.* In prep., Bossi *et al.* Submitted) have shown that these may reach levels of concern for the health and reproductive status of the polar bears. Future investigations will monitor and assess these new problems. In addition cadmium and mercury concentrations will be considered as aetiological factors in the pathogenesis of the variable relations and lesions observed. In collaboration with canadian research groups we are in progress of investigating the relationship between concentrations of organohalogens in different compartments and tissues (liver, brain, kidney, subcutaneous adipose tissue and blood) in the East Greenland polar bear.

## Skulls

No relation between periods of organohalogen pollution and FA in skulls was found. Neither did we find a relation between individual levels of organohalogens and skull FA. On the other hand we found strong indications of period differences in bone mineral density (proposed non-polluted period higher compared to proposed polluted period) and strong negative associations between bone mineral density and various organochlorines (PCBs, DDTs, chlordanes and dieldrin). It cannot be concluded if these are true cause-effect findings but this will hopefully be taken care of in our future investigations of organohalogen toxicity in West Greenland sledge dogs (see next Sections).

## Liver and kidney

In the present study we found signs of chronic inflammation that probably is a result of age, infectious agents and food (lipids) but long-term exposure to anthropogenic toxic substances, such as organohalogens and heavy metals, cannot be ruled out. As we have an ongoing sampling programme in East Greenland we have the possibility of obtaining samples for cytokine investigations and electron microscopy (ultrastructural changes) which is included in the sampling period 2003-2006. In addition, levels of organohalogens will be analysed and compared to liver levels of retinol (vitamin A) as we will continue the investigation of retinol binding protein (RBP) (Heier *et al.* Submitted) in relation to organohalogens and levels of liver vitamin A.

Regarding renal tissue, we will try to dertermine the tubular pigments and see if these have a relation to mercurry accumulation (autometallography) (Woshner *et al.* 2002).

## Reproductive organs

The morphology of both male (testicles) and female (uterus, oviducts and ovaries) reproductive organs will be investigated in relation to reproductive cycles and organohalogen levels. If possible, receptor diagnostics in relation to age, season and pollutants will be conducted.

## Comparative studies of domestic dog (*Canis familiaris*) and Arctic fox (*Alopex lagopus*)

We are conducting a dose-response, case-control study on the Greenland sledge dog. The purpose of the study is to investigate whether the signs of osteopenia in relation to organohalogen body burdens, are true cause-effect interactions. The other main purpose is to find out whether the signs of chronic inflammation in kidney and liver could be due to the organohalogen exposure. Without going into details in the experiment setup, several other biological parameters will be analysed (vaccination response, CYP-isozymes, retinol levels, thyroid homones, sex steroids, cytokines a.o.).

In addition we have a cooperation with Norwegian researchers who investigate the seasonal fasting of the Svalbard Arctic fox. They have an on-going controlled experiment from which we will get samples for analysis and vice versa.

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# Paper I
# Seasonal and temporal trends in polychlorinated biphenyls and organochlorine pesticides in East Greenland polar bears (*Ursus maritimus*), 1990-2001

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## Abstract

Persistent organochlorine (OC) contaminants (PCBs, DDTs, chlordanes (CHLs), dieldrin, hexachlorocyclohexanes (HCHs), chlorobenzenes (CBzs)) were determined in adipose tissue of 92 polar bears (*Ursus maritimus*) sampled between 1999 and 2001 in Central East Greenland (69˚00'N and 74˚00'N, 19˚00'W and 24˚00'W). OC data were presented from subadults (S: females: <5yrs and males: <6 yrs), adult females (F: >5yrs) and adult males (M: >6 yrs). Summed chlorobiphenyl (ΣCBs) concentrations (41 congeners including co-eluters), ΣCHLs and ΣDDTs were the dominant classes of OCs. ΣCBs concentrations were found to be 6470, 8240 and 9100 ng/g lipid weight (lw) i subadults, adult females and adult males respectively. The corresponding figures were: 2010 (S), 2220 (F) and 1710 (M) ng/g lw for ΣCHLs and 462 (S), 462 (F) and 559 (M) ng/g lw for ΣDDTs. The dominant CB congeners were CB153 (32.3%), CB180 (21.4%), CB170 (12.2%) and CB138 (11.0%). Oxychlordane was the dominant (57.1%) CHL-related compound. Concentrations of ΣCBs, ΣCBzs, ΣDDTs, mirex and dieldrin were highest in adult males, whereas concentrations of ΣHCHs were lower than in adult females but not than those in subadults.Adult females had the lowest concentrations of ΣCBzs, mirex and dieldrin. Concentrations of ΣCHLs were lowest in adult males, intermediate in subadults and highest in adult females. ΣCB, ΣHCH and ΣCHL concentrations showed high seasonal variability in adult females but remained relatively constant in adult males and subadults. In general, the OC levels in females appeared to be highest in March and lowest in January or September. Concentrations of ΣCBzs and dieldrin showed seasonal variability in all three groups, with a maximum in March in adult females. ΣCBz concentrations in adult males and subadults of both sexes peaked in April-July, and dieldrin concentrations peaked in April-July in subadults, but not until August in adult males. ΣDDT concentrations increased from January to a maximum in April-July for subadults and in August for adults. Temporal trends within the last decade were examined by comparing the present data to the concentrations reported in samples from 1990 from the same region. ΣCB, *p,p'*-DDE and ΣHCH concentrations in 1999-2001 were 22.1%, 66.3% and 39.3% lower than the 1990 concentrations, respectively. in contrast, ΣCHL and dieldrin concentrations showed differences amongst sex and age groups in the temporal trends, where present concentrations are between 24.4% to 69.3% and 27.0% to 69.0% lower, respectively, relative to the 1990 levels. However, power analy-

sis suggested that firm conclusions could not be drawn regarding the general time trend based on these two sampling periods. The range of half-lives of the various OC classes were estimated to lie between 4.5 and 20.6 years depending on the age and sex groups considered.

*Keywords:* Polar bear, *Ursus maritimus*, East Greenland, organochlorines, OCs, DDTs, PCBs, Chlordanes, Dieldrin, Hexachlorocyclohexane, Chlorobenzenes, seasonal and temporal variation.

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## Introduction

Since the 1950s, large quantities of organochlorine (OC) contaminants such as chlorinated pesticides (dichlorodiphenyltrichloroethanes (DDTs), chlordanes (CHLs), hexachloorcyclohexanes (HCHs), toxaphenes), industrial products (PCBs) and by-products (hexachlorobenzene (HCB), chlorinated dioxins and furans) have been released into the atmosphere in the Northern Hemisphere. The persistence and lipophility of these OCs has resulted in their accumulation in animals in the Arctic marine environment following long-range atmospheric transport from their areas of use in the mid-latitudes of Europe, Asia and North America. The polar bear is one of the most exposed species of the Arctic biota and, in a variety of Arctic marine biota, the highest OC concentrations have generally been found in the East Greenland, Svalbard and Kara Sea areas (de March et al., 1998; AMAP, 2004).

OCs concentrate through Arctic marine foodwebs, and are stored in the adipose tissue due to their high lipophilicity and persistence. Tissues of polar bears (*Ursus maritimus*) contain some of the highest OC levels in the Arctic and the Arctic Monitoring and Assessment Programme (AMAP) has therefore recommended that special attention be paid to this species (de March et al., 1998). Polar bears are reliant on a high fat (lipid) diet derived from their primary prey, ringed seal *(Phoca hispida)*, and to a lesser extent from bearded seals *(Erignathus barbatus)* (Stirling and Archibald, 1977; Smith, 1980). In particular, high levels of OCs are found in polar bear and ringed seals from populations in the eastern Atlantic Arctic (Norstrom et al., 1988; Norheim et al., 1992; Kleivane et al., 1994; Norstrom and Muir, 1994; de March et al., 1998; Norstrom et al., 1998; Muir and Norstrom, 2000; Andersen et al., 2001; Kucklick et al., 2002).

OCs were first detected in polar bears in 1975, in fat samples of individuals taken from Cornwallis Island and southern Hudson Bay, Canada and western Greenland (Bowes and Jonkel, 1975; Clausen and Berg, 1975). Subsequently, OC residues have been detected in polar bears from most Arctic areas (Norstrom et al., 1988; Norheim et al., 1992; Kleivane et al., 1994; Letcher et al. 1995a, 1995b; Letcher et al., 1998; Norstrom and Muir 1994; Norstrom et al., 1998; Muir and Norstrom 2000; Andersen et al., 2001; Kucklick et al., 2002). Overall, concentrations of chlorobiphenyls (CBs) and chlordanes (and metabolites such as oxychlordanes) have been found to be quite high, whilst DDT and HCH are relatively low.

Concentrations of OCs in some individual polar bears from East Greenland and Svalbard were similar to those suspected to have led to a reduction in the

reproductive rates of seals in the Baltic Sea and endocrine disruptions (e.g. Bergman and Olsson 1985; Colborn et al., 1993; Feldman 1995; Bergman 1999; Damstra et al., 2002). Halogenated organic substances such as OCs and their metabolites have been linked to adverse health effects in laboratory mammals, birds as well as wildlife through interactions with immunologic, reproductive and endocrine systems, as well as modulation of subcellular enzyme systems and metabolism (Helle et al., 1976; Safe 1986; Bergman and Olsson, 1985; Tanabe et al., 1987; Safe, 1991; Bergman et al., 1992; Safe, 1994; Swart et al., 1994; Wiig et al., 1998; Bergman, 1999; O'Hara and O'Shea, 2001).

Polar bears mate in March-May (Rosing-Asvid et al., 2001) but active gestation does not commence until September-October, when the pregnant female enters the hibernation den (Ramsay and Stirling, 1988; Wiig et al., 1992). During hibernation (October-March) the female is fasting (for up to 6 months) and is drawing on her fat reserves (Lentfer, 1975). During this period, and until the cubs are born around 1 January (Arnould and Ramsay, 1994), the OCs that have been stored in the female's peripheral tissue (primary fat) are released into the blood stream, which may adversely affect the foetuses *in utero.* The cubs also receive additional OCs via lactational transfer from their mother during suckling (Polischuk et al., 1995, 2002; Takagi et al., 1976; Tanabe et al., 1982; Koppe et al., 1992; Bernhoft et al., 1997). Polar bear milk has an average fat content of ca. 33% (range: ca. 24 to ca. 48%; Jenness et al. 1972), and therefore a high OC load is transferred to the cub during suckling , which may continue for up to 2 years (Arnould and Ramsay, 1994; Oehme et al., 1995). Polar bears may also experience periods when food is scarce or unavailable and, during such periods, the proportion of adipose tissue may be reduced from 50% to 10% of the body mass (Pond et al., 1992; Atkinson and Ramsay, 1995). Hence, the OC load of an individual polar bear not only reflects the initial loads received *in utero* and during infancy, but also the bears sex and age, and the season of the year (Bernhoft et al., 1997; Muir et al., 1999; O'Hara and O'Shea, 2001; Polischuk et al., 1995, 2002). The purpose of the present study was to determine the extent of change in OC concentrations in the fat of East Greenland polar since 1990, and to investigate how OC concentrations vary with age, sex and season. Fat samples were obtained from 92 individual polar bears that were collected as part of the Inuit hunt in central East Greenland during the period 1999-2001. Fat samples were analysed for CBs, DDTs, chlordanes, dieldrin, hexachlorocyclohexanes (HCHs) and chlorobenzenes (CBz).

## Materials and Methods

### Sampling

Fat samples from polar bears were collected by local subsistence hunters in the Ittoqqortoormiit/ Scoresby Sound area in central East Greenland between 69˚00'N and 74˚00'N, 19˚00'W and 24˚00'W in 1999-2001. All tissue samples were taken as soon as possible *post mortem* and stored in separate polyethylene (PE) Whirlpak bags. All samples were kept at outdoor temperature (-5 to -20 ºC) until transferred to a freezer (-10 to -20 ºC). Samples were shipped frozen from Scoresby Sound to Roskilde, where the portion of fat that had been in contact with the PE was trimmed off and the remaining part was transferred to precleaned glass containers with cleaned aluminum foil in between the lid and the glass container. Further storage was at -20 ºC.

### Age Determination

Individual ages were obtained by counting annual growth layer groups in the cementum of the $I_3$ tooth after decalcification, thin sectioning (14 µm) and staining with toluidine blue using the method described by e.g. Hensel and Sorensen (1980) and Dietz et al. (1991). To assure the quality of our readings of GLGs, $I_3$ teeth from four polar bears of known age (5 to 32 years), that had lived in Aalborg Zoo, Denmark, were included in our study. In addition, an intercomparison exercise was conducted using thin-sections ($P_1$) from 23 polar bears that had previously been prepared and their ages estimated by staff of the Canadian Wildlife Service (CWS, Edmonton). For four individuals for which teeth were not available, their age was estimated from the length of the baculum based on a Gompertz "baculum length-on-age" relationship established for 42 aged male specimens from Scoresby Sound (data not shown). These four individuals with baculum lengths of 7.5, 11.1, 19.5 and 20.2 cm were estimated to be 0 (cub of the year), 1.2 (yearling), and more than 10 years old (two animals), respectively.

### Contaminant Analysis

All solvents were of analytical grade or better. Chromatographic materials used for analysis are as follows: Florisil® (magnesium silicate, F100-500, 60-100 mesh) and basic aluminum oxide (Brockman activity grade I, 60-325 mesh), purchased from Fisher Scientific Inc. (Ottawa, Ontario, Canada) and silica gel (Grade 62, 60-200 mesh, 150Å), purchased from Aldrich Chemicals (Milwaukee, WI, U.S.A.). Deactivation of these materials was achieved with double-distilled, *n*-hexane washed $H_2O$. Bio-beads S-X3 (200-400 mesh) were purchased from Bio-Rad Laboratories (Hercules, CA, U.S.A.).

CB and OC standard mixtures were supplied by the Canadian Wildlife Service (Hull, PQ). The compound 1,3,5-tribromobenzene (Accu-Standard Inc., New Haven, CT) was used as the CB/OC internal standard. The method for extraction and clean up of polar bear adipose tissue for OCs and CB analysis has been described elsewhere (Letcher et al., 1995a, 1995b, 1998; Norstrom and Won, 1985; Sandala et al., 2004). Briefly, a ca. 0.5 g sample of polar bear fat was homogenised with sodium sulphate (6:1 ratio by weight), added to an extraction column containing *n*-hexane: dichloromethane (DCM) (1:1), and spiked with the 1,3,5-tribromobenzene internal standard. A 10% portion of the lipid extract volume was used for gravimetric lipid determination. The remaining extract was concentrated and subjected to gel permeation chromatography (GPC) for lipid removal. The contaminant-containing GPC fraction was reduced in volume and subjected to chromatographic clean-up with Florisil® (8.0g, 1.2% deactivated, by weight). Three fractions were collected, i.e., containing CBs (CB#1), most of the organochlorines (OC #1), and OC #2 containing heptachlor epoxide and dieldrin. Each fraction was concentrated to 1 mL for analysis by gas chromatography with micro electron capture detection (GC-µECD). GC-µECD was performed on an Agilent 6890 instrument equipped with a $^{63}$Ni ECD detector and Agilent 7673 automated injector using a fused silica DB-5 GC column [(5% phenyl) methylpolysiloxane (J&W Scientific Inc., Folsom, CA, 30m x 250µm i.d., 0.25µm film thickness). Further details are described in Sandala et al. (2004). Throughout the clean-up procedure, no contaminant-containing fraction was permitted to go to dryness.

An external standard quantification approach was used for quantification of CBs and OCs based on the peak area of the ECD response. ΣCB is the sum of

concentrations of the 41 individual or co-eluting congeners (in order of elution on a DB-5 GC column): CB congeners numbers 31/28, 52, 49, 44, 42, 64/71, 74, 70, 66/95, 60, 101/84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129/178, 182/187, 183, 128, 174, 177, 171/202/156, 200, 172, 180, 170/190, 201, 203/196, 195, 194, 206. CB congeners are numbered according to the corrected IUPAC numbering scheme as described by Guitart et al (1993). ΣDDTs is the sum of *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE. ΣHCH is the sum of the α-, β- and γ-hexachlorocyclohexanes. ΣCHL is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide.

Duplicate analysis (n=9) and method reagent blanks (n=9) were alternately assessed with each batch of 5 polar bear samples. Similar to the procedure of Sandala et al. (2004), the mean CB/OC recoveries as indicated by recovery of 1,3,5-tribromobenzene were 91 ± 7% for all SRM, blank, and polar bear sample and duplicate analysis. CB and OC concentrations were therefore not recovery-corrected. Other than CB congeners and OC compounds, no significant, extraneous positive or negative peaks were observed beyond 5 min. elution time in ECD chromatograms of fractions CB#1, OC#1 and OC#2. The μECD detection limit for OCs and CB compounds was 0.01 ng/g (lipid weight) for polar bear fat. Once analyzed at GLIER, CB#1, OC#1 and #2 fractions were recombined and sent to NWRI to be analysed for selected chlordane components and brominated diphenyl ethers (BDEs) by GC-negative ion chemical ionisation MS (Muir et al., unpublished data).

Standard reference materials 1588a (cod liver oil) and 1945 (pilot whale blubber) supplied by the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) were analysed using identical the same methodology as used for CB/OC analysis in polar bear fat (Hoekstra et al., 2003). Surrogate CB standard recoveries were within the range of 100 - 105% and analyte concentrations were within 10-15% of the certified values. GLIER is certified within the Canadian Environmental Analytical Laboratory (CAEAL) program of the Canadian Standards Association, is a participant in the NCP Quality Assurance Program (Stokker 2003) and participates routinely in interlaboratory comparisons with the National Wildlife Research Centre of the Canadian Wildlife Service.

### Statistical analysis

An analysis of variance (ANOVA) was performed on the lipid content of the fat in order to test whether the fat content differed by season. To normalise the data the square root of the percentages was arcsine transformed prior to the statistical analysis (cf. Zar 1984). The polar bears were sampled between January 1999 and September 2001. Samples taken during the months April to July were pooled because of the low numbers of samples obtained during these months. However, this is the peak period of hyperphagia when fattening occurs prior to the ice break up (Ramsay and Stirling 1988; Messier et al. 1992), which may also provide some biological justification for this pooling. The fat concentration was found to differ significantly between months ($F = 4.16$. $p = 0.002$), with the highest concentration occuring during September and the lowest in the spring months. OC concentrations were therefore expressed as ng/g lipid weight (lw) for presentation of results and further statistical analysis.

Plots of ln-transformed OC concentrations versus age (not shown) did not suggest any clear relationship (e.g. linear relationship) probably due to the

small sample size and the effect of seasonal variation. However, there were tendencies for some compounds to have lower concentrations in females than in males. As no patterns could be detected in the concentrations of OCs relative to age in adult animals as observed in other studies (e.g. Norstrom et al., 1998), the animals were grouped into subadults bears of both sexes (below 5 years for females and below 6 years for males), adult females (5 years old and above) and adult males (6 years old and above) for statistical analysis.

ANOVA with the factors sex and age group (sub adult, adult females and adult males), month (January, February, March, April-July, August and September) and their interactions were performed to test for differences in mean OC concentrations. The least square estimates of the marginal means from the ANOVAs were back transformed to geometric means and plotted to illustrate the differences among months for each sex and agegroup (Fig. 2). Multivariate analysis of variance (MANOVA, Wilks λ) were performed to investigate similarities in the patterns of OC (CBs, DDTs, HCHs, chlordane, chlorobenzene, dieldrin) concentrations among months and sex and age groups and their interactions. Similarities in the patterns among OC were further analysed by reorganising the data prior to ANOVA so that the dependent variable was ln-logarithmically transformed concentrations and the factors were OC compound, month, sex and age groups and all first order interactions. Statistical tests of type III were used for both the ANOVA and the MANOVA.

Data concerning OC concentrations in polar bears from central East Greenland sampled in 1990 (Norstrom et al. 1998) were made available courtesy of R. Norstrom (Dept. of Chemistry Carleton University, Ottawa) so that a comparison could be made of OC concentrations between 1990 and 1999-2001. An ANOVA on ln-logarithmically transformed concentrations, using sex and age group (subadult, adult female and adult males), sampling period and the interactions as factors, was applied to test for differences in mean OC concentrations. The least square estimates of the marginal means from the ANOVAs are used to illustrate the differences between the two sampling periods. For calculation of the half-lives of the analysed contaminants the standard formula:

$$A = A_O^{\frac{-0.693t}{T_{1/2}}}$$

Where $T_{1/2}$ is the half-life, $A_0$ is the concentration at time 0, and A is the concentration after the ellapsed time (t) (Nave 2000). All calculations were performed using the statistical software package SAS® (PC-version V8) and Excel 97® was used as a spreadsheet.

## Results

### Sample Composition

Polar bear tissue samples were collected by local subsistence hunters in the Ittoqqortoormiit/Scoresby Sound area, central East Greenland between 69˚00'N and 74˚00'N, 19˚00'W and 24˚00'W in 1999-2001, as illustrated in Fig. 1. The total sample of 92 polar bears consisted of 50 subadult bears of both sexes, 25 adult females, 16 adult males and 4 with unknown sex or sampling month (Table 1).

**Figure 1**
Capture locations of the polar bears collected between January 1999 and September 2001 included in this study.

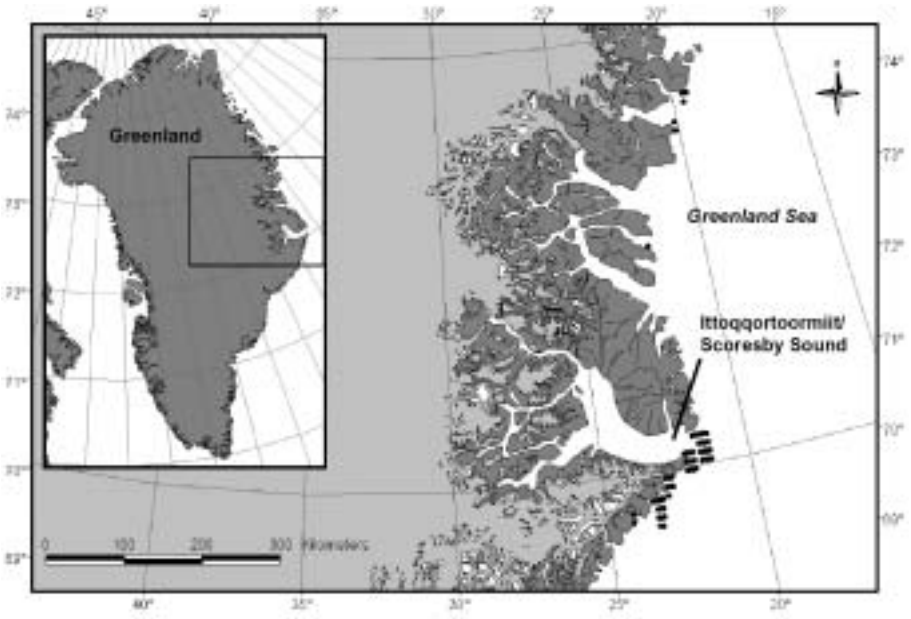


**Table 1**
Number of analysed fat samples from polar bears sampled in Ittoqqortoormiit/Scoresby Sound,East Greenland) in 1999-2001 given by age group, sex and month.

| | Jan | Feb | Mar | Apr-Jul | Aug | Sept | Oct | Unknown | Total |
|---|---|---|---|---|---|---|---|---|---|
| Subadult | 8 | 4 | 6 | 3 | 8 | 19 | 1 | 1 | 50 |
| Adult females | 2 | 4 | 5 | 4 | 2 | 6 | 0 | 2 | 25 |
| Adult males | 5 | 2 | 3 | 1 | 3 | 2 | 0 | 0 | 16 |
| Unknown | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 15 | 11 | 14 | 8 | 13 | 27 | 1 | 3 | 92 |

## OC Concentrations

ΣCBs was the dominant OC compound class, with means from 6470 ng / g lw in subadults to 9100 ng / g lw in adult males, followed by ΣCHLs with means from 1710 ng / g lw in adult males to 2220 ng / g lw in adult females (Table 2). The mean ΣDDT concentrations, ranging from 462 ng / g lw in subadults and adult females to 559 ng / g lw in adult males, were relatively low compared to those of East Greenland ringed seals (prey) which could reflect the ability of polar bears to metabolise DDTs (e.g. Letcher et al., 1995a, 1998; Norstrom et al., 1998, Riget et al. Unpubl. data). Dieldrin exhibited mean concentrations of 208 ng / g in adult females and 245 ng / g lw in adult males, ΣHCH means ranged from 198 ng / g in subadults to 263 ng / g lw in adult females, and ΣCBz means ranged from 100 ng / g adult females to 187 ng / g lw in adult males. Mirex was found at the lowest concentrations, ranging from 2.79 ng / g adult females to 6.59 ng / g lw in adult males.

The large variability (SD) in the OC concentrations of adult females could indicate OCs lipid mobilization from peripheral tissue (primary fat) during reproductive cycles and associated conditions (*e.g.* Polischuk et al., 1995, 2002).

**Table 2**
Mean and SD of organochlorine concentrations (ng/g lw) in polar bears from Ittoqqortoormiit/Scoresby Sound (1999-2001).

| | | ΣPCB | | ΣCBz | | ΣHCH | | ΣDDT | |
|---|---|---|---|---|---|---|---|---|---|
| Group | n | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Subadult | 50 | 6470 | 2980 | 158 | 103 | 198 | 75 | 462 | 227 |
| Adult females | 25 | 8240 | 5820 | 100 | 81 | 263 | 269 | 462 | 324 |
| Adult males | 16 | 9100 | 3560 | 187 | 252 | 218 | 67 | 559 | 441 |
| | | ΣCHL | | Mirex | | Dieldrin | | %Lipid | |
| Group | n | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Subadult | 50 | 2010 | 1110 | 4.08 | 6.20 | 218 | 107 | 92.9 | 5.62 |
| Adult females | 25 | 2220 | 1540 | 2.79 | 5.10 | 208 | 73 | 85.4 | 18.66 |
| Adult males | 16 | 1710 | 763 | 6.59 | 11.20 | 245 | 231 | 84.9 | 7.30 |

**Table 3**
Average percent distribution of the individual PCB congeners, CHLs, DDTs and HCHs in polar bears from Ittoqqortoormiit/Scoresby Sound (1999-2001) based on concentrations in ng/g lipid weight. Based on 92 analysed animals (See Table 1).

| Group | Compound (%) | | | |
|---|---|---|---|---|
| PCBs: | CB31/28 (0.6) | CB52 (0.2) | CB49 (<0.1) | CB44 (<0.1) |
| | CB42 (<0.1) | CB64/71 (0.1) | CB74 (0.5) | CB70 (<0.1) |
| | CB66/95 (0.1) | CB60 (0.1) | CB101/84 (0.3) | CB99 (7.3) |
| | CB97 (0.1) | CB87 (4.8) | CB110 (0.1) | CB151 (<0.1) |
| | CB149 (0.2) | CB118 (1.5) | CB146 (1.2) | CB153 (32.3) |
| | CB105 (0.1) | CB141 (<0.1) | CB179 (<0.1) | CB138 (11.3) |
| | CB158(<0.1) | CB129/178 (0.1) | CB182/187 (0.8) | CB183 (0.9) |
| | CB128 (0.3) | CB174 (<0.1) | CB177 (0.1) | CB171/202* (0.9) |
| | CB200 (0.7) | CB172 (0.2) | CB180 (21.4) | CB170/190 (12.2) |
| | CB201 (0.4) | CB203/196 (0.3) | CB195 (0.1) | CB194 (4.3) |
| | CB-206 (0.9) | | | |
| CHLs: | oxy-CHL (57.1) | t-CHL (0.4) | c-CHL (19.0) | t-nonaCHL (13.9) |
| | c-nonaCHL (0.7) | HEP (8.9) | | |
| DDTs: | p,p'-DDD (2.9) | p,p'-DDE (88.2) | p,p'-DDT (8.9) | |
| HCHs: | α-HCH (24.7) | β-HCH (74,3) | γ-HCH (1.0) | |

* plus /156.

Four CB congeners CB153 (32%), CB180 (21%), CB170 (12%) and CB138 (11%) constituted in total 76% of the total CB concentration (Table 3). Five additional congeners, CB99 (7.3%), CB87 (4.8%), CB194 (4.3%), CB118 (1.5%) and CB146 (1.2%) comprised another 19%, of the total whilst the remaining 32 congeners analysed constituted less than 5% of the total. The dominant chlordane compound was the metabolite *oxy*-chlordane (57%), followed by *cis-c*hlordane (19.0%), *trans-c*hlordane (13.9%) and heptachlor epoxide (8.9%) (Table 3). The metabolite *p,p'*-DDE (88.2%) dominated the ΣΣDDTs followed by *p,p'*-DDT (8.9%), and *p,p*'-DDD constituted the lowest fraction (2.9%) of the ΣDDT. The metabolite β-HCH (88.2%) dominated the ΣHCHs, followed by α-HCH (24.7%) and γ-HCH (1.0%).

**Table 4**
Results of ANOVAs (F-value and significance (p)) of Type III sum of squares with logarithmic transformed concentrations of OC in polar bears from Scoresby Sound (1999-2001) as dependent variable and the factors: Sex, age group and month (M), and their interactions.

| Compound | Age/sex | | Month | | Interaction | |
|---|---|---|---|---|---|---|
| | F | p | F | p | F | p |
| ΣPCBs | 2.29 | 0.109 | 2.13 | 0.061 | 0.87 | 0.561 |
| ΣDDTs | 0.68 | 0.511 | 4.36 | 0.001 | 0.95 | 0.491 |
| ΣHCHs | 0.46 | 0.634 | 4.21 | 0.001 | 2.54 | 0.011 |
| ΣCHLs | 1.00 | 0.372 | 0.57 | 0.752 | 1.15 | 0.336 |
| ΣCBzs | 6.18 | 0.003 | 1.28 | 0.280 | 1.70 | 0.098 |
| Dieldrin | 0.56 | 0.573 | 1.18 | 0.330 | 1.17 | 0.324 |

### OC Concentrations in Relation to Sex, Age and Season

Adult males had highest ΣCBs, ΣCBzs, ΣDDTs, mirex and dieldrin concentrations. In contrast, concentrations of ΣHCHs in adult males were lower than those in females , and concentrations of ΣCHLs were lower than in both subadults and adult females. ΣCBz concentrations differed significantly (at 5% level) among sexes and age groups, whereas ΣHCH and ΣDDT concentrations differed significantly between months. Monthly difference in ΣCB concentration was close to the level of significance (p=0.061) (Table 4). The interaction factor was only significant for HCHs, which indicates a different monthly pattern for the age/sex groups (Table 4). Although month, sex and age group were found to be significant for only a few OC classes, the general pattern of OC concentrations analysed by MANOVA was significantly different between months and between sexes and age groups (Wilks λ, p=0.0002 and p=0.0005, respectively; results not tabulated).

ΣCB, ΣHCH and ΣCHL concentrations showed rather similar monthly patterns, with high variability in adult females and relatively constant levels in adult males and subadults (Fig. 2). The concentrations in females appeared to be highest in March and lowest in January or September. ΣCHLs and dieldrin concentrations showed variability in all three groups, also with a maximum for adult females occuring in March. For ΣCBzs in adult males and subadults the maxima were seen in April-July. Dieldrin concentrations peaked in April-July in subadults, but not before August in adult males. ΣDDT levels increased from January to a maximum in late summer both for adult males, adult females and subadults. This indicates that the concentration of some OC compounds vary seasonally in a similar manner, whilst others do not.

In order to test which OCs varied seasonally in a similar manner, the data was reorganised in such a way that the dependent variable was the logarithmically transformed concentrations and the class variables became the organochlorine compounds, the months and the age/sex groups. An ANOVA was performed including these three factors and all first order interactions. As expected from the previous analysis and deduced from Fig. 2, all main factors and interactions were significant (Table 5). However, when ΣDDTs were removed from the analysis the interaction between month and organochlorine compound was no longer significant (p=0.25, Table 5). This indicated that the monthly patterns seen for the other OCs (ΣCBs, ΣHCHs, ΣCHLs, ΣCBzs and dieldrin) were not significantly different. However, the monthly pattern still differed significantly among sexes and age groups as shown by the significant compound-group interaction.

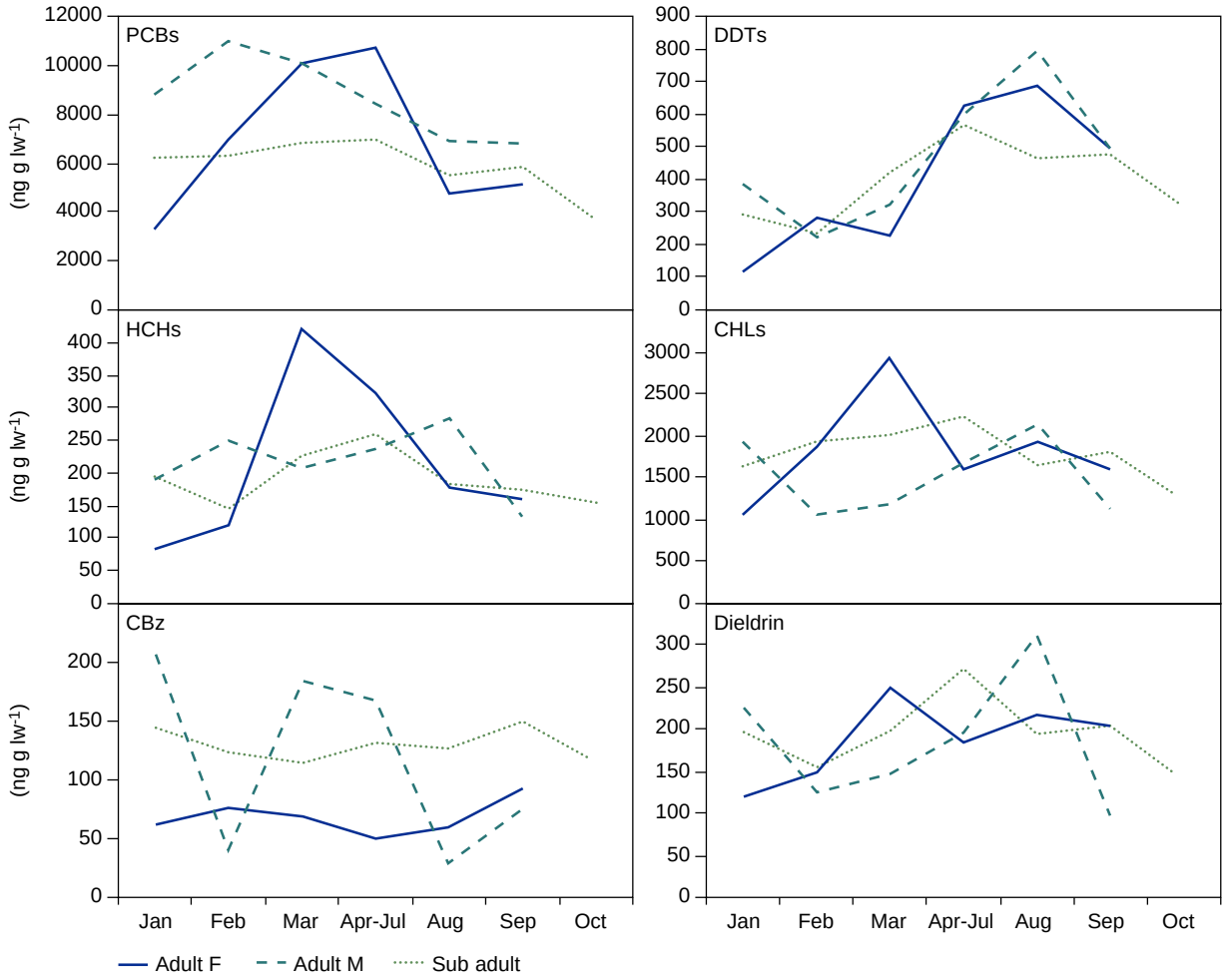


**Figure 2**
Seasonal averages of ∑-PCB, ∑-DDT, ∑-HCH, ∑-CHL, ∑-CBz and Dieldrin (ng/g lw) presented for subadult, adult males and adult females.

**Table 5**
Probability of significance derived from ANOVAs with logarithmic transformed concentrations of OC in polar bears from Scoresby Sound (1999-2001) as dependent variable and the factors: organoclorine compound (C), month (M), sex and age group (G) and their first order interactions.

| | Compound | Month | Age/sex group | Interaction C-M | Interaction C-G | Interaction M-G |
|---|---|---|---|---|---|---|
| All compounds | <0.0001 | 0.0007 | 0.020 | 0.006 | 0.009 | 0.0024 |
| Exluding DDTs | <0.0001 | 0.0033 | 0.025 | 0.252 | 0.0037 | 0.0033 |
| Excluding CBz | <0.0001 | <0.0001 | 0.407 | 0.012 | 0.417 | 0.0001 |

ΣCBz was the only OC contaminant class showing significant differences among sexes and age groups when analysing the compounds separately (Table 4). Generally, ΣCBz concentrations were highest in subadult bears (Fig. 2). Performing the ANOVA on the reorganised data as described in the previous paragraph and removing ΣCBzs from the analysis (instead of ΣDDTs), the interaction between age / sex group and OC compounds was no longer significant (p=0.42, Table 5). This indicated that the sex and age group patterns for the other OC compounds (ΣPCBs, ΣDDTs, ΣHCHs, ΣCHLs and Dieldrin) were not significantly different.

### Long-Term Temporal Changes in OC Concentrations

The following OCs were determined in polar bear fat in both the Norstrom et al. (1998) and the present study: CB congener numbers 99, 149, 118, 146, 153, 138, 183, 180, 170/190 and 194, α- and β-HCH, *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT, oxychlordane, *trans*-nonachlor and heptachlor epoxide, and dieldrin. An ANOVA was performed on selected compound classes, congeners and metabolites with the factors sex and age group, sampling period and their interactions (Table 6). For all OCs, the difference in concentrations between the two periods was highly significant with a significance level of $p<0.0001$, except for *p,p'*-DDE ($p=0.019$) and ΣDDT ($p=0.005$). ΣCHLs and dieldrin were the only compounds for which differences between sex and age group and the "sex/age group-sampling period" interaction were significant at the 5% level. For the other OCs the difference between the two sampling periods showed a similar pattern for juveniles, adult females and adult males. Table 7 shows the proportional differences between the OC concentrations for samples taken in 1990 and 1999-2001. The estimates of concentrations were derived by back transforming the least square means from the ANOVAs. ΣCHLs and dieldrin data are presented for each age/sex group because of the significant interaction factor. The concentrations of individual CB congeners and ΣCB in 1999-2001 were estimated to be 18.8-25.5% of those observed in 1990 (Table 7). The ΣDDT concentrations had not changed to the same degree as ΣCB, and in 1999-2001 they were found at 66.3% of that seen in 1990. *p,p'*-DDE appears to constitute a larger proportion of the ΣDDT, as could be expected due to continued metabolism of the parent compound, and it was present at 71.1% of the concentration seen in 1990. The ΣHCHs concentrations had, on average, decreased to 39.3% of the level seen in 1990, ranging from 35.3% for β-HCH to 54.6% for α–HCH, and indicating half-lives of between 6.7 and 11.5 years. For ΣCHL and dieldrin concentrations the decrease observed between 1990 and 1999-2001 differed between sexes and age groups. In adult females, ΣCHL and dieldrin concentrations in 1999-2001constituted 24.4 and 27.0%, respectively, of those in 1990, whereas the corresponding values for adult males were 68.3 and 69.5% respectively. For subadults of both sexes, the ratios were intermediate to those in adult females and males, with 39.5 and 42.2%, respectively. For comparisons with other studies the percentage decrease and the half-life of each compound/class was calculated. The longest half-lives calculated were for *p,p'*-DDE, dieldrin in adult males and ΣCHLs in adult males (20.6, 19.5 and 17.3 years, respectively). The shortest half-lives were found for CBs and ΣCHLs in adult females and dieldrin in adult females (4.5-5.7, 4.9 and 5.1 years, respectively (Table 7)).

**Table 6**
Results of ANOVAs in polar bears from Scoresby Sound (1999-2001) compared to data from Norstrom et al. (1998) F-values and significance (*p*) of Type III ANOVA sum of squares of logarithmic transformed OC concentrations as dependent variable and the factors: Sex and group, sampling period (year) and their interaction are presented.

| Compound | Age/sex | | Sampling period | | Interaction | |
|---|---|---|---|---|---|---|
| | F | p | F | p | F | p |
| CB99 | 0.70 | 0.497 | 116 | <0.0001 | 0.94 | 0.394 |
| CB153 | 1.59 | 0.208 | 114 | <0.0001 | 1.97 | 0.144 |
| PCB138 | 0.22 | 0.803 | 54.9 | <0.0001 | 0.68 | 0.511 |
| CB180 | 2.05 | 0.133 | 99.0 | <0.0001 | 0.53 | 0.592 |
| CB170/190 | 1.27 | 0.283 | 127 | <0.0001 | 2.06 | 0.132 |
| ΣPCB | 0.76 | 0.471 | 162 | <0.0001 | 1.74 | 0.180 |
| p,p'-DDE | 1.97 | 0.143 | 5.62 | 0.019 | 1.49 | 0.230 |
| ΣDDT | 2.76 | 0.067 | 8.08 | 0.005 | 2.27 | 0.108 |
| α-HCH | 1.79 | 0.172 | 20.8 | <0.0001 | 0.30 | 0.739 |
| β-HCH | 1.73 | 0.182 | 54.1 | <0.0001 | 0.48 | 0.619 |
| ΣHCHs | 1.36 | 0.261 | 48.3 | <0.0001 | 0.39 | 0.678 |
| ΣCHLs | 7.60 | 0.0008 | 46.2 | <0.0001 | 3.71 | 0.027 |
| Dieldrin | 4.71 | 0.011 | 47.9 | <0.0001 | 4.06 | 0.020 |

ΣPCB = sum of 99, 149, 118, 146, 153, 138, 183, 180.,170/190 and 194
ΣHCH = α- and β-HCH
ΣDDT = *p,p'*- DDE, *p,p'*-DDD and *p,p'*-DDT
ΣCHL = oxychlordane, *trans*-nonachlor and heptachlor epoxide

**Table 7**
Percentage of OC left, percentual decrease and calculated half-life of organochlorine concentrations (backtransformed least square means) in East Greenland polar bears sampled in 1990 (data from Norstrom et al., 1998) and 1999-2001 (present study).

| Compound | Left (%) | Decrease (%) | Half-life (years) |
|---|---|---|---|
| PCB-99 | 25.5 | 74.5 | 5.1 |
| PCB-153 | 24.4 | 75.6 | 4.9 |
| PCB-138 | 18.8 | 81.2 | 5.7 |
| PCB-180 | 21.3 | 78.7 | 4.5 |
| PCB-170/190 | 21.5 | 78.5 | 4.6 |
| ΣPCB | 22.1 | 77.9 | 4.6 |
| p,p'-DDE | 71.1 | 28.9 | 20.6 |
| ΣDDT | 66.3 | 33.7 | 17.1 |
| α-HCH | 54.6 | 45.4 | 11.5 |
| β-HCH | 35.3 | 64.7 | 6.7 |
| ΣHCH | 39.3 | 60.7 | 7.4 |
| ΣCHL, sub adult | 39.5 | 60.5 | 7.5 |
| ΣCHL, adult males | 68.3 | 31.7 | 17.3 |
| ΣCHL, adult female | 24.4 | 75.6 | 4.9 |
| Dieldrin, subadult | 42.2 | 57.8 | 8.0 |
| Dieldrin, adult males | 69.5 | 30.5 | 19.5 |
| Dieldrin, adult females | 27.0 | 73.0 | 5.1 |

## Discussion

### Geographical Variation in OC Concentrations

Norstrom et al. (1998) conducted a study of geographical trends in OC concentrations (ΣCBs (16 congeners), *p,p'*-DDE, ΣCHL (11 compounds) and dieldrin) in polar bears around 1990 (samples taken during the period 1989-1993), in which 320 adult bears (5 years of age and older) were sampled from 16 different Arctic locations and analysed within a single laboratory. The highest concentrations were found in bears from Eastern Greenland and in the European Arctic. Concentrations in samples from Canadian locations were intermediate and those in Alaskan polar bears were the lowest observed. Later studies by Andersen et al. (2001) and Lie et al. (2002) have shown that polar bears from Franz Josef Land and the Kara Sea areas of the western Russian Arctic had even higher concentrations of ΣCBs and organochlorine pesticides than those in the Norstrom study. These later studies included data for bears from Svalbard, the Siberian and the Chukchi seas that decreased in the order mentioned. Recent data for CBs (29 congeners), HCHs, CHLs and CHL metabolites, HCB and DDT and metabolites in polar bears from Barrow, Alaska have confirmed that OC concentrations in polar bears from this area are among the lowest in the Arctic (Kucklick et al., 2002).

The geographical trend described earlier has been confirmed by the more recent data. The ΣCB concentrations in adult female polar bears within the present study (mean of 8238 ng/g lw) were considerably higher than the recent 1997-1999 data from Hudson Bay, varying between 2000-3000 ng/g lw (Norstrom 2001; Fisk et al., 2003), even though a clear decrease had taken place during the last ten years. The ΣDDT concentrations in adult female polar bears from East Greenland (EG) (mean of 462 ng/g lw) are also higher than those in animals from Hudson Bay (HB) where average concentrations of 200 ng/g lw in adipose tissue were reported for 1997-1999, and in 2002 (Letcher et al. unpublished data). There were smaller differences between East Greenland and Hudson Bay for ΣCHLs (mean concentrations EG: 2220 ng/g lw; HB: 1700-1800 ng/g lw) and ΣCBzs (mean concentrations EG: 100 ng/g lw; HCB: 60-70 ng/g lw).

### OC Concentrations in Relation to Sex, Age and Season

Adult males were highest in ΣPCBs, ΣCBzs, ΣDDTs, mirex and dieldrin and lowest in ΣCHLs. However, only S-CBzs were found to be significantly different. Similar patterns have been found by e.g. Norstrom (1998), who also found that concentrations of ΣCBs were significantly higher, but those of ΣCHLs lower, in adult males than in adult females. The most probable explanation for the lower concentrations in females are the transference of many OC compounds both transplacentally to the foetus and to the cubs during suckling (e.g. Polischuk et al., 1995, 2002; Bernhoft et al., 1997; Norstrom et al., 1998). The lower concentrations of e.g. ΣCHLs in males are harder to explain, but Polischuk et al. (2002) and Derocher et al. (2003) suggested that sex-specific CHL metabolism was a possible factor in male-female differences. Norstrom et al. (1998) mentioned sex specific dietary differences, the presence of a male specific CYP-enzyme, or the induction of hepatic CYP2B enzyme protein concentrations in liver increasing the degradation rate and so resulting in faster ΣCHLs clearance metabolism in males as possible explanations. Letcher et al. (1996) showed that ΣCHL concentrations in the liver of polar bear from Resolute Bay were strongly correlated with immunologically quantified levels of CYP2B enzyme protein. In other

marine mammals, males also accumulate higher ΣCHL concentrations than females (Muir et al., 1992; Norstrom and Muir, 1994).

In the present study, the subadults showed less variable concentrations than adult males and females for ∑CB, ∑HCH, ∑DDT, ∑CHL, dieldrin and chorobenzene (except compared to adult females for these compounds). The concentrations in adult female peaked in March (∑HCH, ∑CHL) or April-July (∑CB) or August (∑DDT). Chlorobenzene concentrations in adults females remained at a similar level throughout the year. In adult males, peaks in concentrations were found in February for ∑CB, during March and April-July for Chlorobenzene and in August for ∑DDT and dieldrin. The ∑HCH concentrations in adult males remained at a similar level throughout the year. A study of animals from Churchill (western Hudson Bay, Canada) investigated OC concentrations in females and their cubs, subadult and adult males during the fasting period from summer (July-August) to the fall (September-November) (Polischuk et al., 1995, 2002). ∑DDTs declined by 11-50% for most bears during fasting and ∑CHL concentrations declined by 67% during fasting in subadults and adult males, but remained constant in adult females. The overall conclusion of the Canadian study was that ∑DDTs and ∑HCHs declined as the fat deposits became depleted, whereas for ∑CHLs and ∑CBs concentrations generally increased. Their findings regarding ∑DDTs and ∑HCHs were similar to our own, whereas the East Greenland bears also showed a slight decrease in ∑CHL and ∑CB concentrations during the autumn. Differences in the seasonal pattern in OC concentrations between the Western Hudson Bay and East Greenland may be ascribed to the fact that more multi-year ice is present in East Greenland, and hence the bears may not fast for as long as the Hudson Bay bears, as they have longer access to ringed seals hauling out on the ice.

### Long-Term Temporal Changes

All OC compounds in the present study showed a significant decrease in concentrations since 1990, varying from 28.9% to 81.2% for different compounds (representing half-lives of between 4.5 and 20.6 years). Temporal trends of OC concentrations in polar bears have been summarized on the basis of published studies within the Canadian National Assessment and the International AMAP Assessment (Fisk et al., 2003; AMAP, 2004**).** The trends in concentrations of OCs in adult female po-lar bears sampled in the Churchill area from 1968 to 1999 were studied. For several OCs there were no consistent upward or downward trends during the first part of the period from 1968 to 1989. However, ΣCB decreased fairly steadily throughout the 1990s, but with a half-life of approximately 18 years (Fisk et al., 2003). This half-life was considerable longer than was found for the East Greenland polar bears (half-life 4.6 years). The half-life calculated for CB153 in the Canadian study was 19 years, similar to that of ΣCB, whilst the half-life for CB180 was 13 years shorter and of CB-99 was longer (>50 years) than the overall half-life for ΣCB (Fisk et al., 2003). The corresponding values for the East Greenland samples showed less variability and suggested much shorter half-lives (4.5 to 5.7 years; Table 7) indicating a much faster reduction in the contaminant loads in East Greenland polar bears. ΣCB concentrations in Greenland polar bears showed a reduction of 77.9% during the period from 1990 to 1999-2001, whereas less than a factor of 2 difference in ΣCB levels was observed in Hudson Bay through the 1968-1999 period. No long term trend was apparent in animals from Hudson Bay as concentrations in the 1990s were similar to those seen in the late 1960s (Fisk et al., 2003). Temporal trends of OCs have also been studied in polar bears from Svalbard. Henrik-

sen et al. (2001) studied the trend of CB153 concentrations in polar bear blood annually between 1990 and 1998. Decreases of ca. 40% occurred in the early 1990s, and concentrations stabilised thereafter. AMAP, (2004) have estimated an annual percentage decline of CB concentrations in polar bears of to be 2.7% for Hudson Bay polar bears and 6.1% for Svalbard bears, for the period 1989-1999 i.e. 27% and 61%, respectively, over 10 years. The Svalbard values are almost as high as for the East Greenland polar bears, with a reduction of 77.9% over a similar 10 year period. However, Svalbard bears had significantly higher levels of CBs in 1990 than those in Hudson Bay, probably due to the proximity of Svalbard to European sources and air mass movements bringing higher loads of OC to Svalbard compared to Hudson Bay. Hence, PCB levels at Svalbard and in East Greenland may have approached steady state with global distribution of PCBs later than in Hudson Bay because of their proximity to the sources mentioned above. Prior to 1990's, the picture of temporal trends was not quite obvious at Svalbard. Differences in the OC levels measured between 1967 and 1993–1994 ranged from a decrease (CB187) to unchanged concentrations in both sexes (CBs 105,118 and 209) to an increase in females (CBs 99 and 128), to increases in both sexes (CBs 138, 153, 156, 157, 170, 180, 194 and 206 ) (Derocher et al., 2003). The maximum change observed was a nine-fold increase in concentrations of CB157 in adult females. Changes from 1967 to 1993–1994 in contaminant patterns were explained by Derocher et al. (2003) as a combination of selective metabolism and accumulation of organochlorines in polar bears and temporal changes in the contaminant mixture being transported to the Arctic.

Recently organochlorine levels have been determined in ringed seal blubber and shorthorn sculpin liver sampled in 1994, 1999 and 2000 from Ittoqqqortoormiit, central East Greenland (Riget et al., In press). Although these data cover a shorter time span (six years) and year to year variations can be expected, they supplement the findings of the recent changes in concentrations in polar bears and may give indications of the concentrations of OC compounds in the primary food source and at the lower trophic levels of the marine biota. In Ittoqqortoormiit, ΣCB concentrations (10 congeners) in seals (adjusted for age) and sculpins were higher in 1994 than in 1999 but similar to those in the specimens collected in 2000. The difference was only statistically significant in the sculpins.

In southwestern Hudson Bay a significant decrease of ΣDDT concentrations occurred throughout the period from 1968 to the 1990s, after which the level remained constant until 2002 (Norstrom, 2001; Fisk et al., 2003 (and references therein); Letcher et al. unpublished data; AMAP, 2004). Local sources, such as the spraying of DDT for insect control in the local communities and at the large military base at Churchill in the 1950s and 1960s resulted in 2 to 3 times higher ΣDDT levels in polar bear fat in Hudson Bay than in other areas of the Canadian Arctic in 1984. After the DDT ban and the closure of the military base the levels declined in subsequent years. In East Greenland the decrease in concentrations of ΣDDT and *p,p'*-DDE since 1990 was also statistically significant, but this decrease was the lowest observed in the study and so the calculated half-life was the longest (17.1 to 20.6 years) observed. Concentrations of DDTs in seals and sculpin from Ittoqqortoormiit, central East Greenland showed no clear temporal trend from 1994 to 1999 and 2000, but as for the CBs the concentrations of DDTs were lower in 1999 than in the two other years in seals (Riget et al., In press).

The downward trend of ΣHCH concentrations in Hudson Bay polar bears during the 1990s was not significant (Norstrom, 2001), but it became significant when data from 1984 and 1989 were included in the analysis. The half-life calculated for α-HCH in polar bears from Hudson Bay during the 1990s was 10 years, which was slightly longer than that calculated for ΣHCH in East Greenland (7.4 years). In the Canadian sample a decrease in concentration of α-HCH and a consequent increase in β-HCH concentration over the last 30 years were observed. Hence, a significantly higher proportion (50%) of present day ΣHCH in polar bears from Hudson Bay is β-HCH compared to 1984 (25%) and 1968 (17%), whereas the opposite is the case for α-HCH (Fisk et al., 2003 (and references therein); Letcher et al. unpublished data; AMAP, 2004). A similar pattern was observed in ringed seals**.** ΣHCH concentrations also declined in plasma of polar bears from Svalbard between 1991 and 1999 (Lie and Skaare, unpublished data cited in AMAP, 2004). Concentrations were similar between 1991 and 1993, but declined by about 3-fold between 1993 and 1996. Hence, the overall decrease of ΣHCH at Svalbard from 1991 to 1996 is similar to the 2.5 fold decrease observed in East Greenland between 1990 and 1999 to 2001. HCH concentrations in both seals and sculpins from Ittoqqortoormiit, East Greenland showed a decreasing trend, and were significantly higher in 1994 than in 1999 and 2000 in both male seals and sculpins. The proportion of β-HCH in seals from Ittoqqortoormiit was also found to be increasing with time, as would be expected (Riget et al., In press).

ΣCHLs concentrations decreased by between 31.7% and 75.6%, with the values being dependent on the sex and age group studied. Information on time trends is scarce in the literature, but Muir and Norstrom (2000) reported a significant increase in ΣCHL concentrations in polar bears from Davis Strait between 1984 and 1989.

Dieldrin concentrations decreased by between 30.5% and 73.0%, and values were dependent on the sex and age group studied. Also, time trend data for dieldrin are sparse. Muir and Norstrom (2000) reported a significant decrease in dieldrin concentrations in animals from Barrow Strait in the central archipelago, but no apparent changes in those from northern Baffin Bay ΣHCH concentrations in both seals and sculpins from Ittoqqortoormiit showed a decreasing trend with time, and were significantly higher in 1994 than in 1999 and 2000 in both sculpins and male seals. The proportion of β-HCH in seals from Ittoqqortoormiit was also found to be increasing with time (Riget et al., In press).

### Congener Composition

The dominant CB congener CB153 comprised 32% of the ΣCBs, which was somewhat lower than the average (46.0%) for the 1989-1992 bears reported by Norstrom et al. (1998). As the 12 CB congeners included in both the two studies comprised 96.1% of our 41 congeners and 97.3% of Norstroms 16 congeners, the number of congeners analysed could not explain these differences. A summary of previous studies by Fisk et al. (2003) noted a change in the congener composition over time, based on data from the Hudson Bay polar bears. A clear tendency observed was for the proportion of less chlorinated congeners to increase, and for the porportion of the more highly chlorinated congeners to decrease. In the Canadian study, however, the proportion of the recalcitrant congener CB153 showed no time trend, and was stable at around 35% of the total, which is similar to our findings. CB180 comprised 21% of the total Cb concentration in 1999-2001 bears from East Greenland, which is

higher than reported in other studies. Norstrom et al. (1998) found CB180 to constitute 18.5% of the total in 1989-1992 bears from a large part of the Arctic. In a summary by Fisk et al. (2003), CB180 showed a decadal decrease from 17% to 14% of the total, which was the opposite of the trend in East Greenland. A less chlorinated PCB congener like CB99 comprised 7.3% of the 1999-2001 bears from East Greenland, broadly similar to the 8.3% that Norstrom et al. (1998) reported from the trans-Arctic survey of polar bears from 1989-1992. Again this trend was directly opposite to that reported for the Hudson Bay polar bears, where an increase from 10% to 12% was observed over the same decade. Bernhoft et al. (1997) provides less detailed information on the percentage composition of the CB congeners, but the sum of CB153 and CB180 constituted 62% of the ΣCBs, similar to the 64.5% found in our study. A recent study from Svalbard, Franz Josef land, the Kara Sea, the Siberian Sea and the Chuckchi Sea provided percentage compositions of CB153 from 45.4 to 57.5%, of CB180 from 20.4 to 32.1%, of CB99 from 9.3 to 17.9%, of CB194 from 2.5 to 7.6% and of CB118 from 0.6 to 2.0%, similar to our findings of 32.3, 21.4, 7.3, 4.3 and 1.5%, repectively. The simplicity of the PCB congener pattern observed in polar bear fat is consistent with other studies in Canadian polar bear. Letcher et al. (1998) reported similar percent compositions of CB153, CB99, CB138, CB180, etc. in polar bear fat from Resolute Bay bears. The metabolic efficiency of polar bears towards a range of CB congeners, even those with heptachloro- to nonachloro-substitution, is demonstrated by the presence of high levels of HO-PCB and $MeSO_2$-PCB metabolites, which were found in the adipose and whole blood from a sub-set of 19 of the present Greenland polar bears (Sandala et al., 2004), as well as in blood, plasma or adipose tissue from Canadian polar bears (Letcher et al., 1995a, 1995b, 1998 and unpublished results; Wiberg et al., 1998; Sandau et al., 2000; Li and Letcher, 2003). As summarized in Fisk et al. (2003), the proportion of α–HCH was found to be decreasing over time whilst that of β-HCH was increasing. Recently, β-HCH for the Hudson Bay bear was reported to be ca. 50% of the total, which is significantly higher than in 1984 (25%) and in 1968 (17%). We therefore expected to find a similar pattern in East Greenland, but this was, however, not the case, as α–HCH increased from 17.8% to 25.1% and β-HCH showed a decrease from 82.2% to 74.9%. The high percentage of β-HCH is consistent with findings from the Svalbard region where Bernhoft et al. (1997) reported β-HCH to constitute 81% of the ΣHCH. The dominant CHL compounds were the metabolite *oxy*-chlordane (57%) followed by *cis*-chlordane (19.0%), *trans*-chlordane (13.9%) and heptachlor Epoxide (8.9%). Bernhoft et al. (1997) reported an even higher percentage of *oxy*-chlordane as 72% was given as the average sum of chlordanes. Henriksen et al. (2001) investigated the minimum yearly sampling strategy required to detect a significant temporal trend by the use of power analysis based on the natural variability observed between years. The conclusion from this study was that it would be unlikely to detect significant trends with less than 7 or 8 years of sampling if the annual trend was 5%, and suggested a target of 10 - 25 samples a year and suggested blood plasma as the preferred matrix of contaminant analysis. However, a recent paper by Lydersen et al. (2002) concludes that blood is a poor substrate for monitoring OC concentrations relative to blubber samples in harp seals (*Pagophilus groenlandicus*). During a fasting period, the sum ΣCB concentrations in blubber samples remained unchanged, whereas blood levels in two out of three seals showed an increase of 720% during a 28 days fasting period. On the other hand adipose fat can still show substantial variability, as the use of blubber samples from ringed seals of 2 to 4 years of age from Greenland waters suggests that 13 years of sampling and analysis may be necessary to detect an annual trend of 10% with a power of 90% at a 5% significance level (Riget et al., 2000).

## Conclusions

Adult males were highest in ΣCBs, ΣCBzs, ΣDDTs, mirex and dieldrin. In contrast, adult males had lower concentrations than females for ΣHCHs and for ΣCHL, and also lower concentrations than subadults for ΣCHL. Seasonal variation in persistent OC concentrations is considerable and this must be taken into account in the design of monitoring programs for the study of spatial and temporal trends. The degree of seasonal variation is dependent on the analysed compound. ΣCB, ΣHCH and ΣCHL concentrations showed somewhat similar monthly patterns, with high variability in adult females and relatively constant levels in adult males and subadults. The levels in females appear to be highest in March and lowest in January or September. ΣCBzs and dieldrin showed variability for all three analysed groups, with a similar maximum for concentrations in adult females in March. For ΣCBzs in adult males and subadults the peak appeared in April-July, and dieldrin concentrations peaked in April-July for subadults, but not before August for adult males. ΣDDT concentrations increased from January to a maximum in late summer for adult males, adult females and subadults. A significant decrease in OC concentrations was observed for all contaminants, age and sex groups studied over the period from 1990 to 1999-2001. However, no firm conclusions should not be drawn based on two sampling periods due to the year to year variability. The rate of decrease in concentrations is more rapid than that observed over the same period in polar bears from Canada, but similar to that seen in polar bears from Svalbard.

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# Paper II
# Trends in fluctuating asymmetry in East Greenland polar bears (*Ursus maritimus*) from 1892 to 2002 in relation to organohalogen pollution

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## Abstract

Fluctuating asymmetry (FA) was studied in skulls of 283 polar bears (*Ursus maritimus*) sampled in East Greenland from 1892 to 2002. Fourteen metric bilateral traits in skull and lower jaw were measured and compared between polar bears born before 1961 (*n*=94) and after 1960 (*n*=189). The period 1892-1960 was chosen to represent a period prior to appearance of organohalogens (PCBs, DDTs, HCHs, CHLs, HCB, PBDEs and dieldrin) originating from long-range transport to East Greenland from southern latitudes. The period 1961-2002 represents the period where polar bears have been exposed to organohalogens. During this latter period the level of organochlorines is believed to have increased from 1960 to the late 1980-ies followed by a likely decrease from 1990 to 2002. Within this later period other compounds such as *e.g.* polybrominated flame retardants are believed to have increased throughout the period. Two different analysis showed, that the degree of fluctuating asymmetry did not differ statistically between the two periods in ten of thirteen traits. In fact, when significant differences were found in four of the traits, the fluctuating asymmetry was lower in skulls sampled after 1960. The degree of fluctuating asymmetry was higher in adults than in subadults for 6 of the 13 traits, whereas a higher degree of fluctuating asymmetry was found for only one trait in one of the analysis for subadults relative to adults. Females had a higher degree of fluctuating asymmetry than males in one trait. A time trend analysis did find fluctuations over time for five traits but the relationship was weak as the trend appeared to occur by chance due to the high number of regressions analysed (*n*=42). A correlation analysis of FA versus the sum concentrations of various classes of organohalogens in adipose tissue from a subsample of 94 recently collected polar bears (1999-2002) showed no significant trend either. Hence, the present study could not document a relationship between skull asymmetry in polar bears and periods with different exposure to organohalogens. These findings are possibly influenced by nutritional status, genetic factors, a subeffect exposure of organohalogens or confounded by other environmental factors (*e.g.* temperature) within the two investigated periods.

*Key words:* polar bear, *Ursus maritimus*, fluctuating asymmetry, organohalogens, DDTs, PCBs, stress, endocrine disruption.

## 1. Introduction

Environmental (*e.g.* pollution and infections) and genetic stress (*e.g.* bottlenecks) may affect the ability of an individual to address developmental instability and thereby disruption or modulation of its "true" phenotype and fitness (Palmer and Strobeck 1986, Møller 1996, Møller and Swaddle 1997, Rus Hoelzel et al., 2002). Often developmental instability is expressed as asymmetry between bilateral traits, and when measured in a group of individuals (population) the instability is called *fluctuating asymmetry* (FA) (*Ibid.*). In it's definition "FA refers to random differences that occur between right and left sides in bilateral traits" and these differences reflect "mistakes" in developmental processes that result from the inability of the genotype to effectively buffer itself against environmental perturbations" (Van Valen 1962). FA is defined as the small, intermittently occurring difference between a left and a right trait, where the side with the largest trait and the magnitude of the difference shifts randomly. Fluctuating asymmetry is measured as left / right metric and meristic differences. In FA the differences observed are usually small and cannot be categorised as malformations (Jagoe and Haines 1985, Palmer and Strobeck 1986, Jones 1989, Leary and Allendorf 1989).

In addition to FA, *directional asymmetry* (DA) and *antisymmetry* (AS) have also been discovered (Jagoe and Haines 1985, Palmer and Strobeck 1986, Jones 1989, Leary and Allendorf 1989). DA occurs when the larger side is consistent (*e.g.* the right testicle / ovary is larger than the left in humans). In AS the largest side varies equally between left and right (*e.g.* the larger signalling claw of male fiddler crabs is the left and right side with same frequency), but this type of asymmetry occurs very rarely.

FA has been measured in invertebrates (*e.g.* flies and crabs) and vertebrates (fish, birds and mammals) (Jagoe and Haines 1985, Palmer and Strobeck 1986, Jones 1989, Leary and Allendorf 1989) and used as an environmental stress indicator in numerous studies of wildlife and laboratory mammals (*e.g.* Palmer and Strobeck 1986, Nachman and Heller 1999). Correlations between *in utero* induced FA and temperature / noise extremes, limited food access and quality and chemical contaminants have been reported (*e.g.* Siegel and Doyle 1975a-c, Doyle et al., 1977, Siegel et al., 1977a). Exposure to anthropogenic contaminants such as PCBs (PolyChlorinatedBiphenyls), DDTs (DichloroDiphenylTrichloroethanes), HCHs (HexaCycloHexanes), CHLs (CHLordanes), HCB (HexaChloroBenzene), PBDEs (PolyBrominated-DiphenylEthers) and dieldrin have been suspected as an environmental stress factor that can lead to endocrine disruption through agonism and / or antagonism of hormone-dependent processes in different target organs and tissues involved in endocrine functions (Bergman and Olsson 1985, Colborn et al., 1993, Swart et al., 1994, Feldman 1995, de March et al., 1998, Bergman 1999, Damstra et al., 2002, AMAP, 2004). Interference with receptors in the main endocrine pathway results in endocrine disruption and stress through the hypothalamus---hypophysis---target organ / tissue axis leading to elevated blood corticosteroid levels (adrenocortical hyperplasia; Cushing's Syndrome) and may therefore also induce FA (Bergman and Olsson 1985, Colborn et al., 1993, Feldman 1995, Borisov et al., 1997, de March et al., 1998, Bergman 1999, Damstra et al., 2002).

Metric size difference between bilateral traits of homologous cranial bones and teeth and meristic bilaterally traits (foramens *i.e.* openings in the skull for nerves and blood vessels) have been used to describe FA and developmental instability (Jagoe and Haines 1985, Palmer and Strobeck 1986, Leary and Allendorf 1989). FA in skulls has been used to reveal stress potentially induced by organohalogens in populations of marine mammals such as *e.g.* Baltic grey seal (*Halichoerus grypus*), harbour seal ( *Phoca vitulina*) and california sea lion (*Zalophus californianus*) (Zakharov and Yablokov 1990, Bergman et al., 1992, Mortensen et al., 1992, Schandorff 1997a-b, Coy and Schaeff 2001) and fish (Valentine and Soulé 1973, Valentine et al., 1973, Ames et al., 1979, Jagoe and Haines 1985).

Polar bears (*Ursus maritimus*) from East Greenland, Svalbard and the Kara Sea carry higher loads of organohalogens than polar bears elsewhere in the Arctic (*e.g.* Norstrom et al., 1988; Bernhoft et al., 1997; Norstrom et al., 1998, Andersen et al., 2001, AMAP, 2004, Lie et al., 2003; Dietz *et al.* 2004; Letcher et al. unpublished data). Levels of PCBs in polar bears from East Greenland and Svalbard are in the range believed to negatively affect reproduction and survival of seals in the Baltic Sea (Bergman and Olsson 1985, Feldman 1995, de March et al., 1998, Bergman 1999, AMAP, 2004). There have been several recent reports on polar bears from Svalbard, and relationships between blood-circulating levels of sex hormones and PCB and other organochlorines. For example, Σ-PCB concentrations (sum of 16 congeners) were found to make significant contributions to the variation in plasma testosterone levels in 121 male Svalbard bears (Oskam *et al.* 2003). In female Svalbard bears, progesterone was found to be positively correlated with Σ-PCBs (Haave et al., 2003). In Svalbard bears of varying sex and age (1991-1994), plasma retinol concentrations and the ratio of total T4 to free T4 decreased linearly with increasing concentrations of Σ-PCBs (Skaare et al., 2001) and studies have also indicated strong associations between Σ-PCBs and immunotoxicity (Bernhoft et al., 2000, Lie et al., 2004, Lie et al., submitted). It is therefore imperative that the significance of the possible effects of environmental persistent pollutants on polar bears is understood to implement effective conservation strategies.

In the present study, we investigated and compared FA in East Greenland polar bears in samples collected during the period 1892-1960 relative to the period 1961-2002. The date of 1960 was somewhat arbitrary, but represents a time point the contrasts a substantial change in organohalogen loadings into this Arctic region. A number of organohalogens were put into commercial use in the early 1950-ies, and there was an estimated delay of 5-10 years before a significant signal of these contaminants, originating and transported from lower latitudes, could be detected in the tissues of high trophic predators such as polar bears in East Greenland (de March et al., 1998, Norstrom et al., 1998, AMAP, 2004). In this study we compared the FA in skulls of 94 polar bears sampled before 1960 with FA in skulls of 189 sampled after 1961. Finally our recent collection of samples allowed us to examine a potential relationship between individual levels of organohalogens and FA in 94 of the polar bears collected between 1999-2002.

## 2. Materials and methods

### 2.1 Sampling and preparation

A sample of 283 polar bear skulls from East Greenland originating from an area between Skjoldungen at 63º15'N and Danmarks Havn at 76º30'N was studied. A total of 178 skulls had been collected by expeditions and local hunters in the period 1892-1987 and stored at the Zoological Museum University of Copenhagen, Denmark. These were the East Greenland polar bear skulls with known collection year (death of individual) that could be located to the present investigation. In connection with a study to determine effects of pollutants on polar bears in East Greenland, a total of additional 105 skulls were sampled from the Inuit´s subsistence hunting between 1999 and 2002. All skulls with lower jaw were macerated and boiled gently (< 10 min.) so muscles and tendons could be removed prior to $H_2O_2$ oxidation for 24 to 48 hours. For the temporal comparisons the 283 skulls were devided into bears collected before 1960 (*n*=93) and bears collected after 1960 (*n*=189).

Samples of subcutaneous adipose tissue from 77 of the polar bears were collected by local subsistence hunters in the Ittoqqortoormiit/ Scoresby Sound area in central East Greenland between 69˚00'N and 74˚00'N, 19˚00'W and 24˚00'W in 1999-2001. All tissue samples were taken as soon as possible *post mortem* and stored in separate polyethylene (PE) Whirlpak bags. All samples were kept at outdoor temperature (-5 to -20 ºC) until transferred to a freezer (-10 to -20 ºC). Samples were shipped frozen from Scoresby Sound to Roskilde, where the portion of fat that had been in contact with the PE was trimmed off and the remaining part was transferred to precleaned glass containers with cleaned aluminum foil in between the lid and the glass container. Further storage was at -20 ºC.

### 2.2 Measurements for fluctuating asymmetry

Fourteen metric bilaterally traits were examined to measure the degree of fluctuating asymmetry in all 283 skulls (Fig. 1 and Table 1). Bilaterally length and distances were measured with a calliper to the nearest mm (trait 25, 28) or 0.1 mm (traits 21, 22, 24, 29, 30, 31, 33, 34, 35, 36, 37 and 38). To estimate the measurement error (ME) a double determination was conducted on skull # 852, 853, 854, 856, 857, 860 and 861. However, these data were found to be insufficient and therefore a 10 time measurement on skull # 851 was conducted as well. All measurements used to estimate ME were done on two different days by the same investigator. Finally, in most of the polar bears, a reduced number of measurements were taken due to skull damage from gunshots in connection with the killing.

**Figure 1**
Specific skull locations of the 14 measurements taken for the study of fluctuating asymmetry in East Greenland polar bears (Ill.: M. Kirkegaard). See Table 1 for description of measurements.

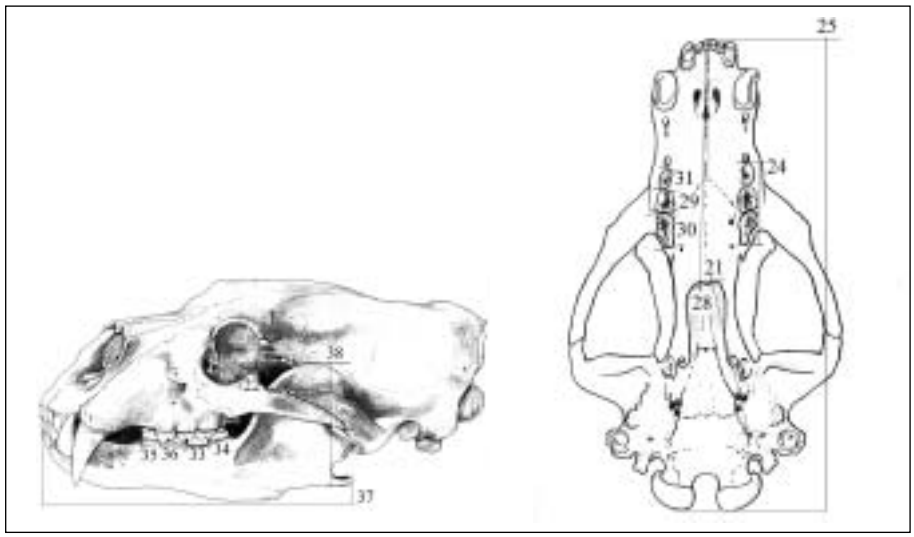


**Table 1**
Detailed anatomical description of the traits used in the present study of fluctuating asymmetry in East Greenland polar bears. Each trait is given a number and divided on skull and lower jaw. The measurements are viewed in Fig. 1. I: incisor, P: pre molar in upper jaw, M: molar in lower jaw, p: pre molar in lower jaw and m: molar in lower jaw.

| Trait | Anatomical definition of distance measurements |
|---|---|
| Skull | |
| 21 | The minimal distance between the midpoint of the posterior margin of the hard palate to the anterior margin of the jugular foramen. |
| 22 | The minimal distance between the margin of the zygomatic process and the margin of the postorbital process. |
| 24 | The minimal distance between the posterior margin of the P4 crown to the anterior margin of the M2 crown. |
| 25 | The maximal distance between the anterior margin of the I1 crown and anterior margin of the occipital condyles. |
| 28 | The minimal distance from the anterior margin of I1 crown to the midpoint of the posterior margin of the hard palate. |
| 29 | The minimal distance between the anterior and posterior margin of the M1 crown. |
| 30 | The minimal distance between the anterior and posterior margin of the M2 crown. |
| 31 | The minimal distance between the anterior and posterior margin of the P3 crown. |
| Lower jaw | |
| 33 | The minimal distance between the anterior and posterior margin of the m1 crown. |
| 34 | The minimal distance between the anterior and posterior margin of the m2 crown. |
| 35 | The minimal distance between the anterior and posterior margin of the p2 crown. |
| 36 | The minimal distance between the anterior and posterior margin of the p3 crown. |
| 37 | The maximal distance between the anterior margin of the mandibular symphysis and the posterior margin of the angular process. |
| 38 | The maximal distance between the margin of the angular process and the coronoid process. |

## 2.3 Age Determination

The age determination was carried out by counting the cementum Growth Layer Groups (GLG) of the lower right incisor ($I_3$) after decalcification, thin sectioning (14µm) and staining (Toluidine Blue) using the method described *e.g.* by Hensel and Sorensen (1986) and Dietz et al., (1991).

## 2.4 Contaminant analysis

### 2.4.1 PCBs and OCs

Polar bear adipose tissue samples (*n*=77) were analysed for PCBs (Poly-ChlorinatedBiphenyls), DDTs (DichloroDiphenylTrichloroethanes), HCHs (HexaCycloHexanes), CHLs (CHLordanes), HCB (HexaChloroBenzene) and dieldrin according to Sandala et al. (2004) and Dietz et al. (2004) at the Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada. An external standard quantification approach used for PCBs and OCs in the adipose tissues was based on peak area of the GC-µECD response, which is described in detail in Dietz et al. (2004). Briefly, sPCBs is the sum (s) of the concentrations of the 51 individual or co-eluting congeners (if detected): CB # 31/28, 52, 49, 44, 42, 64/71, 74, 70, 66/95, 60, 101/84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129/178, 182/187, 183, 128, 174, 177, 171/ 202/156, 200, 172, 180, 170/190, 201, 203/196, 195, 194, 206. sDDTs is the sum of 4,4'-DDT, 4,4'-DDD and 4,4'-DDE. sHCHs is the sum of the α-, β- and γ-hexachlorocyclohexane. sCHLs is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. Contaminant fractions were subsequently sent to the National Water Research Institute (Environment Canada, Burlington, Ontario, Canada L7R 4A6 (NWRI)) for determination of brominated diphenyl ether (PBDE) flame retardants.

### 2.4.2 PBDEs

PBDE (*n*=78) were determined by electron capture negative ion (low resolution) MS using an external standard. Briefly, PBDEs is the sum (s) of the concentrations of the 35 individual or co-eluting congeners (if detected): BDE# 10, 7, 11, 8, 12/13, 15, 30, 32, 28/33, 35, 37, 75, 71, 66, 47, 49, 77, 100, 119, 99, 116, 85, 155/126, 105, 154, 153, 140, 138, 166, 183, 181, 190. Gas chromatographic conditions for the PBDEs were described by Luross et al. (2002).

## 2.5 Statistics

The statistical analysis were performed with the SAS statistical software package (SAS V8) and a significance level of $p$=0.05 was used, except where stated otherwise. The magnitude of fluctuating asymmetry was estimated from the absolute value in the distance difference between right and left side (L-R) measurements. It was decided that (L-R) >5mm were excluded from the analysis as the macroscopic investigation evaluated these as being malformations rather than true FA (Jagoe and Haines 1985, Palmer and Strobeck 1986, Jones 1989, Leary and Allendorf 1989).

#### 2.5.1 Normality

The values of (L-R) were tested for following the normal distribution by Shapiro-Wilk test. Furthermore, skewness and kurtosis were tested by *t*-test (Zar 1984) in order to assess deviations from normality.

#### 2.5.2 Size dependency

Both linear regression analysis and Spearman correlation analysis were performed to test for size dependency between FA magnitude (L-R) and trait size (skull size). In cases where a significant (at the 5% level) size dependency was detected, it was attempted to normalise the absolute value of (L-R) by size in order to remove the size dependency and obtain normality.

#### 2.5.3 Measurement error

It is of major importance to account for measurement error (ME) when studying FA (Merilä and Björklund 1995). The basic of calculating the measurement errors (ME) were repeated measurements and first the measurement errors were calculated as the mean coefficient of variation (CV) (*Ibid.*). In the FA analysis the measurement errors were expressed as the variance of the error term derived from a one-way single-factor analysis of variance (ANOVA) with "individuals" as the factor (*Ibid.*). The average of the repeated measurements were used in the further analysis.

### 2.5.4 Statistical analysis of directional and fluctuating asymmetry

The variance of FA in the periods (1892-1960 vs. 1961-2002) and in age/sex groups (subadults, adult males and adult females) were derived by performing two-way mixed ANOVAs using "skull side" as fixed factor and "individual" as random factor. The error term from the two-way ANOVA was an estimate of the variance of FA plus ME (Palmer and Strobeck 1986, Merila and Björklund 1995). The variance of FA was then derived by subtracting the estimate of ME from the repeated measurements. The difference in FA between time periods (1892-1960 vs. 1961-2002) and between adult males, adult females and subadults were finally tested by *F*-test.

The two-way mixed ANOVA also provided a test of significant directional asymmetry ("skull side" factor). Besides this test, a two-tailed, one-sample *t*-test for mean=0 for (L-R) was employed to analyse for the significance of directional asymmetry in the 14 traits investigated.

A non-parametric Kruskall-Wallis test was used to test differences in the absolute value of FA between periods and age/sex groups. This test was done to supplement the results from the two-way ANOVA, because the normality of data could be doubtful in several cases.

#### 2.5.5 Polynomial regression

Temporal trends in FA was also analysed by third order polynomial regression analysis (absolute value of FA versus year of birth) were conducted for each trait (and for adult males, adult females and subadults, separately) in order to explore the development of FA from 1892-2002. Before analysing; the age of the 283 bears was subtracted from collection year to define the year of birth of the animals and hence to relate the individual bears to the period, where a possible *in utero* disturbance/disruption in relation to de-

velopmental instability (*i.e.* the magnitude of FA) could have been initiated (*e.g.* Siegel and Doyle 1975a-c, Doyle et al., 1977, Siegel et al., 1977a). The model was successively reduced for non-significant interactions ($p$>0.05) and the significance was evaluted from the remaining reduced model.

#### 2.5.6 FA versus contaminants

For the 94 samples from 1999 to 2002 of which both skulls and contaminant analysis were available a non-parametric Spearman correlation coefficient was used to test for significant correlations between levels of individual organohalogens and FA. Due to the large numbers of tests ($k$=98) a Bonferroni correction of the $p$-estimates was applied to avoid the generation of significant results simply by chance. The critical alpha level was set to $p$=0.05 / 98=0.0005.

## 3. Results

### 3.1 The sample

Before analysing the trend of FA between periods (before and after 1960) and age / sex groups, individuals were grouped as being adult males (≥6 years), adult females (≥5 years) and the remaining individuals of both sexes as subadults (Table 2). The sample consisted of a total of 283 individuals distributed as 142 subadults, 78 adult males and 63 adult females (Table 2). The number of skulls and age of individuals were equally distributed over time with few peaks, although the period 2000-2002 was over-represented (Fig. 2).

**Table 2**
Age, sex and number of polar bear skulls divided on periods. The number of subadults and females differ in particular between two periods investigated.

| Period/group | Subadults | Females | Males | Sum |
|---|---|---|---|---|
| 1892-1960 | 45 | 13 | 37 | 94 |
| 1961-2002 | 97 | 50 | 41 | 189 |
| Sum | 142 | 63 | 78 | 283 |

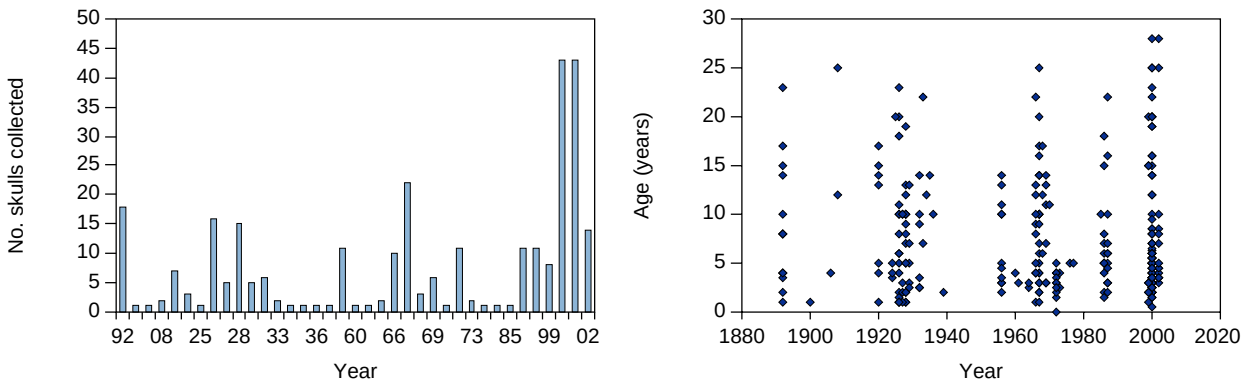


**Figure 2**
Number of skulls collected per year from 1892 to 2002 (n=283) (left) and their individual age (right).

### 3.1.1 Normality

Basic statistics for the 14 FA traits are shown in Table 3. Deviation from normality could be detected in 12 of the 14 traits (Table 4). To further investigate the deviations from normality the data was analysed for skewness and kurtosis (Table 4). Asymmetric distributions (skewness) could be significantly detected in 3 traits; two traits (29 and 32) were skewed to the right and one trait (28) was skewed to the left side. Kurtosis were found significant in 11 traits, and in all cases the distribution was leptokurtic (a distribution having many values around the mean and in the "tails", far from the mean) (Table 4). The 3 significant traits from test for skewness showed divergent and no consisting left-right trends against a single side. As the assumption of normality of the data in several cases could be doubted, both parametric and non-parametric statistical tests were performed in further analysis.

**Table 3**
Basic statistics (millimeter) of the 14 metric bilateral traits used to detect fluctuating asymmetry in polar bear skulls (n=289) 1892-2002. L: left; R: right; SD: Standard Deviation; n=count.

| Trait/statistics | Mean ± SD | | Min-Max | | n |
|---|---|---|---|---|---|
| | L | R | L | R | |
| Skull | | | | | |
| 21 | 110.4 ± 14.8 | 110.3 ± 13.9 | 21-154.3 | 68.4-154.6 | 247 |
| 22 | 26.4 ± 2.7 | 26.3 ± 2.6 | 18.3-33.5 | 18.4-34.9 | 260 |
| 24 | 58 ± 4.6 | 58.4 ± 4.2 | 24-67.1 | 29.3-66.9 | 259 |
| 25 | 325.6 ± 38.8 | 326.3 ± 33.3 | 25-392 | 210-391 | 227 |
| 28 | 166.9 ± 18.9 | 167.6 ± 16.9 | 28-207.5 | 110-207 | 268 |
| 29 | 19.2 ± 1.3 | 19.2 ± 1.1 | 12.3-29.0 | 12.4-21.6 | 275 |
| 30 | 25.5 ± 2.5 | 25.7 ± 2.5 | 12.9-30.4 | 13.9-30.9 | 274 |
| 31 | 15.2 ± 1.8 | 15.0 ± 1.5 | 12.5-31.0 | 11.9-25.9 | 260 |
| Lower jaw | | | | | |
| 33 | 19.6 ± 1.5 | 19.6 ± 1.3 | 13.4-33.0 | 13.7-28.3 | 266 |
| 34 | 14.9 ± 1.9 | 14.8 ± 1.5 | 11.0-34.0 | 10.3-18.4 | 230 |
| 35 | 12.5 ± 1.8 | 12.6 ± 1.0 | 10.5-35.0 | 10.8-21.5 | 252 |
| 36 | 20.5 ± 1.6 | 20.4 ± 1.2 | 11.9-36.0 | 11.9-23.1 | 249 |
| 37 | 217.5 ± 24.8 | 217.6 ± 21.6 | 154.7-266.2 | 154.4-264.3 | 244 |
| 38 | 89.0 ±1 2.8 | 89.3 ± 12.3 | 56.6-115.1 | 56.1-116.5 | 240 |

### 3.1.2 Size dependency

In trait 25, 31 and 38 statistically significant size dependency (L-R increases by skull size) could be detected and for trait 30, the regression showed significant level just above 5% and the Spearman correlation a level just below 5% (Table 4). When normalising for size, this dependency disappeared for trait 25 and partly for 38 but not 30 and 31 and therefore it was decided to work with size normalised FA (L-R / (L / 2+R / 2)) in case of trait 25 and 38 in the further analysis.

**Table 4**
Results (test and p-values) from normality and regression tests of traits. t-test (mean=0): test on (L-R), Shapiro-Wilk: test on (L-R), Skewness: test on (L-R), Kurtosis: test on (L-R), Regression analysis: regression of │L-R│ on size and Spearman correlation: correlation of │L-R│ and size. '*': indicates statistic significant differences at the p=0.05 level, '**': indicates statistic significant differences at the p=0.01 level and'***': indicates statistic significant differences at the p=0.001 level.

| Trait/test | t-test | | Shapiro-Wilk | Skewness | | Kurtosis | | Regression | Spearman | |
|---|---|---|---|---|---|---|---|---|---|---|
| | t | p | | t | p | t | p | p | p | df |
| Skull | | | | | | | | | | |
| 21 | 3.51 | <0.01** | <0.01** | -0.19 | 0.21 | 0.98 | <0.01** | 0.63 | 0.14 | 255 |
| 22 | 2.47 | 0.014* | 0.02** | 0.01 | 0.95 | 1.15 | <0.01** | 0.07 | 0.21 | 271 |
| 24 | -5.56 | <0.01** | 0.01** | 0.10 | 0.50 | 1.11 | <0.01** | 0.62 | 0.76 | 272 |
| 25 | 134.42 | <0.01** | <0.01** | -0.16 | 0.31 | 0.81 | 0.01* | 0.02* | 0.05* | 240 |
| 28 | -4.68 | <0.01** | <0.01** | -0.87 | <0.01** | 2.77 | <0.01** | 0.08 | 0.02* | 282 |
| 29 | -3.72 | <0.01** | <0.01** | 0.45 | <0.01** | 1.78 | <0.01** | 0.78 | 0.82 | 291 |
| 30 | -4.59 | <0.01** | <0.01** | -0.15 | 0.30 | 2.70 | <0.01** | 0.06 | 0.04* | 289 |
| 31 | 2.31 | 0.022* | <0.01** | 0.24 | 0.11 | 0.66 | 0.03* | 0.03* | <0.01** | 273 |
| Lower jaw | | | | | | | | | | |
| 33 | -2.68 | <0.01** | <0.01** | 0.59 | <0.01** | 1.05 | <0.01** | 0.33 | 0.20 | 281 |
| 34 | 0.37 | 0.709 | 0.02* | 0.11 | 0.48 | 0.71 | 0.02* | 0.40 | 0.91 | 245 |
| 35 | -8.1 | <0.01** | <0.01** | -0.18 | 0.23 | 0.22 | 0.46 | 0.23 | 0.28 | 267 |
| 36 | -2.51 | 0.013* | <0.01** | 0.05 | 0.74 | 1.53 | <0.01** | 0.31 | 0.68 | 265 |
| 37 | 5.03 | <0.0001*** | 0.061 | -0.23 | 0.13 | 0.18 | 0.56 | 0.102 | 0.20 | 254 |
| 38 | -2.18 | 0.0301* | 0.62 | -0.09 | 0.56 | 0.19 | 0.54 | <0.001*** | <0.01** | 253 |

Deviation from normality was detected in all skull traits and four lower jaw traits. Skewness was found in two skull traits and one lower jaw test and kurtosis was found significant in all skull traits and three lower jaw traits. There was a size dependency for trait 25, 31 and 38 however, when normalising for size this dependency disappeared for trait 25 and partly for 38 and therefore it was decided to work with size normalised FA (L-R/(L/2+R/2)) in case of these traits.

#### 3.1.3 Measurement error

The CV ranged from 0.17% for trait 38 to 1.27% for trait 35, the same magnitude as CV in similar studies (Merila and Björklund 1995). The estimates of measurement error (ME) and FA expressed as variances derived from the ANOVAs of the two periods and three age/sex groups are presented in Table 5. ME ranged from 0.007 for trait 35 to 0.646 for trait 37 when excluding the size-normalised traits.

### 3.2 Statistical analysis of asymmetry

#### 3.2.1 Directional asymmetry

First a two-tailed one-sample *t*-test for mean=0 for (L-R) showed that in 13 of 14 traits the mean differed significantly at the 5% level from 0 (indicating directional asymmetry), however, whether the right or the left side was largest was *not* consistent (Table 4). Secondly the two-way ANOVA found that in 9 (21, 24, 25, 28, 29, 30, 35, 36 and 37) of the 14 traits statistical significant directional asymmetry was present. For the traits (24, 28, 29, 30, 35, 36) the right measurement was significantly higher than the left, while for the traits (21, 25, 37) the opposite was the case. As for the above mentioned *t*-test the dominating side was not consistent and all 14 traits were therefore used and evaluated in the present investigation of FA.

### 3.2.2 Fluctuating asymmetry

For trait 25 in the period after 1960 and in subadults and for trait 33 in females, the ME exceeded the degree of FA plus ME and consequently these data were excluded from the *F*-test exploring differences in FA between periods and age / sex groups (Table 6). In traits 21, 22, 24, 30, 31, 34, 35, 37 and 38 the ME were less than 50% of the FA in all 5 groups, whereas in traits 28, 29, 33 and 36 the ME in two to four of the 5 groups exceeded 50% of the FA (Table 5).

The results of *F*-tests of differences in FA expressed as variance between periods (before and after 1960) and between age / sex groups are viewed in Table 6. For five (21, 22, 29, 33, 37) out of a total of 14 traits, the fluctuating asymmetry before 1960 was significantly higher than after 1960. Only for trait 28 a significant difference between females and males (females higher than males) could be detected. In three traits (21, 22, 29) the FA in females was significantly higher than in subadults, and for six traits (21, 22, 29, 33, 36, 38) the FA was higher in males than in subadults.

When assessing differences in the absolute value of FA by Kruskal-Wallis test between periods and age / sex groups, three traits (22, 29, 33) showed significantly higher FA in bears from before 1960 than after 1960, similar to the results of the *F*-tests (Table 6 and 7). However, also traits (25, 38) showed significantly higher FA before 1960 analysed by the Kruskal-Wallis test, which was not detected by the *F*-tests. Finally, trait 35 showed significantly lower FA before 1960, which was not detected by the *F*-test either. When testing for differences between age and sex groups by Kruskal-Wallis tests, only traits 24 and 38 showed significantly differences although not in agreement with each other as the orders subadults>males>females and males>subadults>females were found in the two comparisons respectively (Table 7). For trait 38 the result of the Kruskal-Wallis test was in agreement with the *F*-test where males showed a significant higher degree of FA compared to subadults while the result for trait 24 was not in agreement with the *F*-test.

**Table 5**
Variances of measurement error (ME) and fluctuating assymetri (FA) estimated from ANOVAs within periods and age/sex groups. FA: (L-R); $FA_2$: FA variances from ANOVA. ‡: ME>ME+$FA_2$. Trait 25 and 38 is normalised by (L-R/(L/2+R/2)).

| Trait/period and group | | 1960 | >1960 | Adult females | Adult males | Subadults |
|---|---|---|---|---|---|---|
| | ME | ME+$FA_2$ (df) | ME+$FA_2$ (df) | ME+$FA_2$ (df) | ME+$FA_2$ (df) | ME+$FA_2$ (df) |
| Skull | | | | | | |
| 21 | 0.388 | 2.840 (64) | 1.546 (141) | 2.468 (54) | 2.185 (67) | 1.371 (83) |
| 22 | 0.182 | 1.402 (63) | 0.611 (150) | 0.879 (57) | 1.317 (69) | 0.454 (86) |
| 24 | 0.261 | 0.508 (63) | 0.722 (149) | 0.470 (60) | 0.660 (65) | 0.819 (86) |
| 25 | $9.58*10^{-6}$ | $1.05*10^{-5}$ | $8.78*10^{-6}$‡ | $1.10*10^{-5}$ | $1.04*10^{-5}$ | $7.44*10^{-6}$ ‡ |
| 28 | 0.233 | 0.412 (71) | 0.425 (148) | 0.439 (60) | 0.268 (73) | 0.539 (85) |
| 29 | 0.028 | 0.101 (73) | 0.037 (155) | 0.063 (62) | 0.080 (75) | 0.037 (90) |
| 30 | 0.065 | 0.433 (71) | 0.520 (154) | 0.371 (61) | 0.532 (76) | 0.554 (87) |
| 31 | 0.014 | 0.105 (63) | 0.086 (148) | 0.087 (61) | 0.092 (639 | 0.092 (86) |
| Lower jaw | | | | | | |
| 33 | 0.033 | 0.064 (67) | 0.043 (153) | 0.024 (60)‡ | 0.069 (70) | 0.049 (90) |
| 34 | 0.055 | 0.225 (44) | 0.172 (146) | 0.164 (54) | 0.211 (619 | 0.178 (74) |
| 35 | 0.007 | 0.061 (59) | 0.065 (148) | 0.052 (60) | 0.078 (60) | 0.064 (86) |
| 36 | 0.032 | 0.052 (60) | 0.052 (145) | 0.044 (59) | 0.068 (57) | 0.046 (88) |
| 37 | 0.646 | 3.315 (62) | 1.620 (143) | 2.403 (60) | 1.965 (61) | 2.013 (82) |
| 38 | $7.66*10^{-6}$ | $1.21*10^{-4}$ | $1.18*10^{-4}$ | $1.06*10^{-4}$ | $1.52*10^{-4}$ | $1.04*^{10-4}$ |

In case of trait 25 in the period after 1960 and in subadults and for trait 33 in females, the ME exceeded the degree of FA plus ME and consequently these data were excluded from the further statistical analyses.

**Table 6**
Results from F-tests (p-values) of the differences in the magnitude of FA between periods and age/ sex groups. "↓" indicate that FA is larger in the period 1892-1960 compared to 1961-2002, is larger in adult females compared to males, is larger in adult females compared to subadults and is larger in adult males compared to subadults. Note that the comparisons for trait 25 and comparisons including females of trait 33 has been excluded from the analysis due to that measurement error exceeds FA. '***': statistical significant differences at the p=0.001 level.

| Trait/period and group | 1892-1960 vs. 1961-2002) | Females vs. Males | Females vs. Subadults | Males vs. Subadults |
|---|---|---|---|---|
| Skull | | | | |
| 21 | <0.001*** ↓ | 0.28 | 0.001*** ↓ | <0.001*** ↓ |
| 22 | <0.001*** ↓ | 0.97 | <0.001** ↓ | <0.001*** ↓ |
| 24 | 0.99 | 0.99 | 0.99 | 0.99 |
| 28 | 0.62 | <0.001*** ↓ | 0.95 | 0.99 |
| 29 | <0.001*** ↓ | 0.95 | <0.001*** ↓ | <0.001*** ↓ |
| 30 | 0.84 | 0.96 | 0.97 | 0.60 |
| 31 | 0.13 | 0.60 | 0.60 | 0.50 |
| Lower jaw | | | | |
| 33 | <0.001*** ↓ | | | <0.001*** ↓ |
| 34 | 0.05 | 0.91 | 0.68 | 0.16 |
| 35 | 0.62 | 0.96 | 0.83 | 0.17 |
| 36 | 0.49 | 0.99 | 0.73 | <0.001*** ↓ |
| 37 | <0.001*** ↓ | 0.13 | 0.14 | 0.55 |
| 38 | 0.44 | 0.93 | 0.46 | <0.04*** ↓ |

The results in this Table 6 shows that FA was higher in the proposed non-polluted period (1892-1960) when compared to the proposed polluted period (1961-2002). These results are supported by the non-parametric tests showed in Table 7.

**Table 7**
Kruskal-Wallis test results (p-values) for the analysed traits between the two time periods and between the three sex/age groups. "↓":FA larger during the period 1892-1960 compared to 1961-2002. "↑": FA larger during the period 1961-2002 compared to 1892-1960. "†":FA increase in the order: subadults> males>females and for "‡" increase in the order: males>subadults>females. '*': statistic significant differences at the p=0.05 level and '**': statistic significant differences at the p=0.01 level.

| Trait/period and group | 1892-1960 vs. 1961-2002 | Subadults vs. males vs. females |
|---|---|---|
| Skull | | |
| 21 | 0.89 | 0.10 |
| 22 | 0.04* ↓ | 0.99 |
| 24 | 0.70 | 0.045* † |
| 25 | 0.03* ↓ | 0.40 |
| 28 | 0.77 | 0.28 |
| 29 | <0.01* ↓ | 0.16 |
| 30 | 0.87 | 0.42 |
| 31 | 0.89 | 0.76 |
| Lower jaw | | |
| 33 | <0.01* ↓ | 0.14 |
| 34 | 0.37 | 0.53 |
| 35 | 0.02* ↑ | 0.80 |
| 36 | 0.38 | 0.88 |
| 37 | 0.20 | 0.43 |
| 38 | 0.02* ↓ | <0.01* ‡ |

The results in this Table 7 shows that FA was higher in the proposed non-polluted period (1892-1960) when compared to the proposed polluted period (1961-2002) except for trait no. 35.

Third order polynomial regressions (intercept, 1st, 2nd and 3rd order) describing the development over time (1892-2002) in the absolute magnitude of FA (L-R) for subadults, adult males and adult females, separately, showed non-significant influences of the 1st, 2nd and 3rd order variables in nine of the fourteen traits indicating no time trends. The exception from this was trait 22 (adult males), 29 (subadults), 33 (adult females), 37 (subadults) and 38 (adult females). For trait 22 and 33 the intercept, 1st and 2nd order variable were significant (all: $p<0.01$) and the best estimated model fitted the 2nd order regression (parabola) with absolute values of FA decreasing from 1892 to around 1960 and increasing in the period 1960 to 2002. For trait 29 and 37 all variables were significant (all: $p<0.05$) and the best estimated model fitted a 3rd order regression where the absolute values of FA increases from 1892 to around 1925, declines from around 1925 to 1980 and increases slightly from 1980-2002. In the regression of trait 38 all links were significant (all: $p<0.04$) and a 3rd order regression model with a twice slope shift fitted the absolute FA values from 1892-2002 best. Opposite for trait 29 and 37 the trait values declines from 1892 to around 1930 and increases from 1940-1990 following a slightly decline from around 1990 to 2002.

### 3.3 Changes over time in fluctuating asymmetry

Based on the *F*-test a significant difference were found for trait 21, 22, 29, 33 and 37 between the two periods with the pre-pollution period being higher in FA compared to the pollution period. The Kruskal-Wallis test also showed the same significant difference between the two different periods in trait 22, 25, 29 and 33.

For trait 21, which was not normal distributed, the significance obtained by the *F*-test may be doubtful. In case of trait 37, which did not deviate significantly from normality, the *F*-test showed significantly difference between the two periods opposite to the result of the Kruskal-Wallis test. Additionally the Kruskal-Wallis test found a significant difference between the period in trait 25 (not normal distributed) with the prepollution period having the highest FA. As the test result for trait 25 was not in accordance with the *F*-test this result could be doubted. The Kruskall-Wallis test also found a higher FA in trait 35, which was not normal distributed, in the pollution period compared to the pre-pollution period. This test result was not in accordance with the general picture nor the *F*-test and could be explained by the non-normal distribution of this trait (as for trait 21) or that it resulted by chance due to the large ($n$=14) number of traits examined. Therefore a significant period difference with the prepollution period (1892-1960) carrying a higher FA compared to the post-pollution period (1961-2002) could be found in trait 22, 29, 33 and 37 while the results for trait 21 and 25 indicating the same difference were more doubtfull.

In the description of the development in FA over the entire period 1892 to 2002 only five cases of the regression variables (1st, 2nd or 3rd) were found to be significant. No consistent patterns between the five traits could be found and it was therefore concluded that these results likely resulted by chance due to the large ($n$=14) number of traits examined within the three age and sex groups (42 tests).

### 3.4 FA versus contaminants

Levels of contaminants (ng / g l.w.) of PCBs (51 IUPAC congeners), DDTs (*p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT), HCHs (α-HCH, β-HCH, λ-HCH), CHLs

(oxy-chlordane, *trans*-chlordane, *cis*-chlordane), HCB, PBDEs (35 IUPAC congeners) and dieldrin in the recently collected polar bears from the years 1999 through 2002 are viewed in Table 8A. It is seen that sum (Σ) PCBs and dieldrin constitute the highest levels followed by Σ-DDTs, Σ-HCHs and dieldrin while HCB and Σ-PBDEs carry the lowest levels. Further details on these levels in relation to season, sex and age are given in Dietz et al., (2004).

Table 8B gives the results from the analysis from the Spearman correlation of individual FA versus contaminant concentrations. All of the correlation coefficients were low and non-significant but for trait 31 this was significant negatively correlated to DDTs and for trait 35 this was significant negatively correlated to PCBs. On the other hand trait 37 was significant positively correlated to Σ-PCBs. However, none of these three correlation results were significant after Bouferroni correction of the *p*-estimates (Table 8.b).

**Table 8A**
Basic statistics of organohalogen compounds (OHCs) analysed in the present study (levels in ng/g l.w.). SD: Standard Deviation; n=count. PCBs and Chlordanes were the predominating groups while DDTs, HCHs and Dieldrin were rather low. HCB and PBDEs were the lowest contaminant concentrations analysed.

| Variable/statistics | Mean ± SD | Min-max | n |
|---|---|---|---|
| ∑–PCBs | 6444 ± 3236 | 897-20407 | 77 |
| HCB | 77 ± 66 | 2-331 | 77 |
| ∑–HCHs | 194 ± 123 | 14-818 | 77 |
| ∑–DDTs | 391 ± 215 | 73-1113 | 77 |
| Dieldrin | 185 ± 81 | 26-490 | 77 |
| ∑–CHLs | 1395 ± 1016 | 243-7465 | 77 |
| ∑–PBDEs | 55 ± 32 | 17-196 | 78 |

**Table 8B**
Spearman correlation coefficients between concentrations of individual OHCs and magnitude of FA traits (L-R). Non significant differences are not indicated while '$^{*}$': indicates statistic significant differences at the p=0.05 level; '$^{**}$': indicates statistic significant differences at the p=0.01 level and '$^{***}$': indicates statistic significant differences at p=0.001 level. '$^{\dagger}$': indicates that the results were no longer significant after Bonferroni correction (*k*=98).

| Trait/ Variable | 21 | 22 | 24 | 25 | 28 | 29 | 30 | 31 | 33 | 34 | 35 | 36 | 37 | 38 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ∑–PCBs | -0.01 | 0.11 | 0.18 | 0.01 | -0.003 | 0.07 | -0.1 | -0.14 | 0.08$^{\cdot}$ | -0.11 | -0.25$^{*,\dagger}$ | -0.07 | 0.26$^{*,\dagger}$ | 0.16$^{\cdot}$ |
| HCB | 0.13 | -0.09 | 0.13 | -0.02$^{\cdot}$ | -0.02 | 0.2 | -0.004 | -0.01 | 0.06 | -0.16 | -0.002 | 0.13 | 0.17 | 0.03 |
| ∑–HCHs | 0.07 | -0.07 | 0.12 | -0.07 | -0.02 | 0.15 | -0.05 | -0.14 | -0.01 | -0.01 | -0.04 | 0.02 | 0.11 | 0.08 |
| ∑–DDTs | 0.07 | -0.01 | 0.06 | 0.02 | 0.09 | 0.06 | 0.12 | -0.35$^{***,\dagger}$ | -0.01 | -0.11 | -0.08 | -0.01 | 0.03 | -0.03$^{\cdot}$ |
| Dieldrin | 0.05$^{\cdot}$ | -0.11 | 0.04 | -0.01 | -0.07 | -0.02 | 0.01 | -0.16 | 0.02 | 0.02 | -0.2 | 0.1 | 0.16 | 0.01 |
| ∑–CHLs | 0.04 | -0.13 | 0.05 | -0,14 | -0.002 | -0.14 | -0.11 | -0.08 | 0.05 | 0.004 | -0.2 | -0.02 | 0.18 | -0.02 |
| ∑–PBDEs | 0.13 | -0.12 | -0.04 | 0.18 | -0.03 | -0.12 | -0.1 | -0.05 | 0.04 | 0.13 | -0.03 | -0.07 | -0.08 | 0.02 |

# 4. Discussion

## 4.1 Changes over time in fluctuating asymmetry

The overall finding in the present study was that developmental instability existed for some of the 14 traits measured, with respect to fluctuating asymmetry (FA) in the sampled skulls of polar bears from East Greenland during the period 1892-2002. In general, input of various air- and water-born organochlorines into the study area from around 1960, was not reflected in an increase of FA in the polar bear skulls. Earlier studies of marine mammals have detected differences in developmental instability over time and correlated these to decades of pollution. Schandorff (1997a), who investigated fluctuating asymmetry in Kattegat harbour seal (*Phoca vitulina*) collected in the period 1889-1988 ($n$=61), found a period difference between FA and fractal dimensions (suture measurements) in some traits but not in all. Five of 20 *F*-tests conducted on foramens showed a significant higher degree of FA in the polluted period compared to the non-polluted period (we did not measure FA in foramens in the present study). In the same study three of 12 *F*-tests conducted on teeth (upper 3rd molar) showed significant higher degree of FA in the polluted period compared to the non-polluted period while this was not the case in our present study on polar bears (*i.e.* trait 30). In Table 9, levels of Σ-PCBs and Σ-DDTs in the Kattegat harbour seal (blubber) before / around 1988 is compared to the levels in bear in the present study. For Σ-PCBs, the levels are comparable to the lower levels of the Kattegat harbour seal before 1988, while for Σ-DDTs the level was 2-10 times higher and the thresshold of FA was not reached (subeffect exposure).

**Table 9**
Range in the levels of organohalogenes (µg/g l.w.; blubber) linked to fluctuating asymmetry in the Kattegat harbour seal (Phoca vitulina) and Baltic grey seal (Halichoerus grypus) (range for juveniles, subadults and adults) from before and around 1988 compared to the range in levels of polar bears from East Greenland in the present study. n: number of observations (data from: Blomkvist et al., 1992; Schandorff 1997a,b and Zakharov and Yablokov 1990). It is viewed that the contaminant concentrations in the present polar bears are significant lower compared to the Kattegat and Baltic seals.

| Species/variable | Organohalogen compound (n) | Concentration around 1988 | Concentration in adipose tissue of East Greenland polar bears in the present study (n) |
|---|---|---|---|
| Kattegat harbour seal | ∑–PCBs (38) | 6-110 (blubber) | 1-20 (77) |
| Kattegat harbour seal | ∑–DDTs (38) | 2.0-13 (blubber) | 0.1-1.1 (77) |
| Baltic grey seal | ∑–PCBs (37) | 32-5300 (blubber) | 1-20 (77) |
| Baltic grey seal | ∑–DDTs (37) | 11.0-1600 (blubber) | 0.1-1.1 (77) |

Zakharov and Yablokov (1990) investigated 24 bilateral meristic traits (mainly foramen) in Baltic grey seals (*Halichoerus grypus*) ($n$=50) to compare a pre-pollution and a pollution period. In 11 of the 24 traits they found a significant increase from the non-polluted period to the polluted (in the present study we did not measure the FA of foramen). The concentrations of Σ-PCBs and Σ-DDTs in the Baltic grey seal around 1988 compared to the present polar bear sample are viewed in Table 9. Here it is seen that the concentrations in the grey seal exceeds 10-1000 folds the concentrations in the polar bears and thereby the effect exposure of FA of foramen.

Also Pertoldi et al. (1997) investigated developmental stability in the Eurasian otter (*Lutra lutra*) collected 1861-1994 (*n*=172). They measured three metric traits of the skull and one on the lower jaw of which we measured the one in the lower jaw and two of the three in the skull. Of these; FA in three traits in females and two in males had increased significantly by time and it was stated that this was probably due to lower genetic variations (bottle necks) over time rather than toxic levels of contaminants (although levels of contaminants was measured these were not reported; see section FA versus contaminants). FA in skulls of the Yellowstone grizzly (*Ursus arctos*) has also been associated with genetic isolation in 16 traits measured (Picton et al., 1990) but this association is not likely in the present polar bear sample as a relatively constant hunting has taken place over the last century (Sandell et al., 2001).

FA is expected to be a result of *in utero* disturbances (*e.g.* Siegel and Doyle 1975a-c, Doyle et al., 1977, Siegel et al., 1977a-b). Therefore FA in polar bears can be explained by environmental factors other than organohalogens. Noise, temperature extremes and food availability are some environmental factors impacting FA (*e.g.* Siegel and Doyle 1975a-c, Doyle et al., 1977, Siegel et al., 1977a-b, Nilsson 1994, Carrascal et al., 1998). These results from controlled studies of laboratory mammals (rats) have shown a significant correlation between audiogenic and temperature stressors and dental and bone fluctuating asymmetry. If these factors differ between the two periods 1892-1960 and 1961-2002 it could explain that FA in the period before 1960 is higher than in the period after 1960. Higher climatic fluctuations (temperature extremes) in the first period could explain food availability and thereby a high degree of developmental instability in the polar bears compared to the second period. However, a temperature effect is not likely either as temperatures above normal have been experienced in East Greenland during the last two decades. Furthermore, an added complexity is that temperatures were also relatively high in this area between *ca.* 1930 and *ca.* 1960 (Førland 2002). Finally genetic stress (bottleneck) could differ between the periods although this is not likely as a relatively constant hunting has taken place over the last century and no clear change has been observed in the number of bears obtained or the areas where the hunt has taken place (Sandell et al., 2001).

### 4.2 Age and sex differences

In general, FA was higher in adults than in subadults and was in the *F*-test significant in 3 out of 14 traits for females and in 6 out of 14 traits for males (both distance and teeth measurements). This result may be doubted as all traits except 38 was not normal distributed and that the result was only supported by the non-parametric Kruskall-Wallis in trait 38. Only for one trait (28) FA were found higher in females compared to males and this result was not significant in the Kruskall-Wallis test. The Kruskall-Wallis test found that subadults were slightly significant higher than adults in trait 24 but as it was not in accordance with the *F*-test the result may be doubted. In general it may be postulated that it has resulted by chance due to the large (*n*=14) number of traits examined between three groups (42 tests).

Although FA is thought to be a result of *in utero* disturbances (*e.g.* Siegel and Doyle 1975a-c, Doyle et al., 1977, Siegel et al., 1977a-b) one may speculate whether different age and sex groups have different FA. These results are in accordance with the finding in harbour seals (Schandorff 1997a-b), where a higher degree of fluctuating asymmetry in foramen FA (but not in teeth) was

detected in adults compared to subadults, as well as in adult females compared to adult males. Pertoldi et al., (1997) found no differences in FA between subadults and adult Eurasian otter. Males usually have higher body burdens of organohalogens than females, and older animals usually higher than subadults (Bernhoft et al., 1997, Norstrom et al., 1998, Dietz et al., 2004). Our analysis detected differences between sex (females higher than males) in one trait. Schandorff (1997a-b), found a higher degree of FA in foramen FA in adult females compared to adult males and Pertoldi et al. (1997) unfortunately did not test the difference between sexes of the Eurasian otter.

Swaddle et al. (1994) pointed out two potential problems with investigating FA in museum samples; one is that the collection of skulls could be biased (in our situation this could mean extraordinary old animals or asymmetric skulls) and secondly it is important to differentiate between "true" FA and FA as a result of wear and damage. Therefore it could be speculated whether the skulls sampled in the period before 1960 mainly by people interested in collecting "trophie" bears that were biased towards larger (older) animals, rather than skulls from individuals that were collected after 1960. The majority of the animals collected after 1960 was made up by the samples from 1999-2002, which was considered representative of the Inuit´s catch from that period. In the analysis we excluded measurements as a result of wear and damage as well as large left-right differences so the present investigation should not be influenced by such a bias.

### 4.3 FA versus contaminants

The significant correlation of trait versus contaminants did not show a clear pattern as two traits (*i.e.* trait 35, 31) were negatively correlated to Σ-PCBs and Σ-DDTs while one (*i.e.* trait 37) was postive correlated to Σ-PCBs. These significant correlations probably occured by chance due to the large number of correlations ($n$=98) investigated, and due to the large individual variability in the contaminant levels. Meanwhile FA likely resulted from prenatal *in utero* disruptions, rather than being related to contaminant exposure at the time of sampling. Few previous studies of mammals have linked FA to organohalogen contaminant concentrations on an individual by individual level. Pertoldi et al., (1997) examined such correlations (DDTs and PCBs), but thet did not find a relationship between FA and individual contaminant burdens in Eurasian otter. The authors explained the lack of correlation by the high individual variability of organohalogens including seasonal patterns and sex differences.

## Conclusions

The present study of polar bear skulls ($n$=284) from East Greenland did not reveal a relationship between developmental instability (*i.e.* fluctuating asymmetry; FA) and time periods of contrasting concentrations of organohalogens. Clear differences between the pre-pollution (1892-1960) and pollution (1961-2002) time periods were found in 4 of 13 traits and these showed significant higher degree of FA in the *pre*pollution period compared to the pollution period. An analysis of FA over the entire period 1892 to 2002 did not show a clear pattern either. Differences in FA between age / sex could be found in 6 of 13 traits measured with the adults carrying the highest degrees of FA. In one trait, females had a higher degree of developmental instability than males. Finally a correlation of FA versus individual contaminant concentrations could not show any clear trend. Hence, the present

study could not document a relationship between skull asymmetry in polar bears and periods with different exposure to organohalogens possibly due to either genetic factors, a subeffect exposure of organohalogens or confounding by other environment factors (*e.g.* temperature) within the two investigated periods.

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# Paper III-a
# Is bone mineral composition disrupted by organochlorines in East Greenland polar bears (*Ursus maritimus*)?

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*Key words:* Polar bear, *Ursus maritimus*, PCBs, DDTs, CHLs, dieldrin, endocrine disruption, osteoporosis, bone mineral density, BMD.

## Abstract

Bone mineral density (BMD) in polar bear (*Ursus maritimus*) skulls ($n$=139) from East Greenland sampled during 1892-2002 was analysed. The primary goal was to detect possible changes in bone mineral content (osteopenia) due to elevated exposure to organochlorine (PCBs, DDTs, CHLs, dieldrin, HCHs, HCB) and PBDE compounds. To ensure that the BMD in skull represented the mineral status of the skeletal system in general, BMD in femur and three lumbar vertebrae were compared in a subsample. Highly significant correlations between BMD in skull and femur ($r$=0.99; $p$<0.001; $n$=13), and skull and vertebrae ($r$=0.97; $p$<0.001; $n$=8) were detected. BMD in skulls sampled in the supposed pre-organochlorine and PBDE period (1892-1960) was significantly higher than in the supposed pollution period (1961-2002) for subadult females, subadult males and adult males (all: $p$<0.05) but not adult females

(p=0.94). Negative correlation between organochlorines and the skull BMD was in subadults found for ∑-PCBs ($p<0.04$) and ∑-CHLs ($p<0.03$) and in adult males for dieldrin ($p<0.002$) and ∑-DDTs ($p<0.02$) (indications for ∑-PBDEs in subadults; p=0.06).In conclusion, the strong correlative relationships suggested that disruption of the bone mineral composition in East Greenland polar bears may have been caused by organochlorine exposure.

## Introduction

Bone mineral composition in mammals is based on a complex set of interrelated mechanisms, and is influenced by various nutritional and environmental factors (*e.g.* Ganong 1991; Johansson and Melhus 2001; Johansson et al. 2002; Leder et al. 2001; Michaelsson et al. 2003; Promislow et al. 2002; Sarazin et al. 2000). Furthermore, environmental stressors such as exposure to harmful chemicals, starvation, temperature extremes and noise have been shown to disrupt bone mineral composition in laboratory mammals (Bergman and Olsson 1985; Brandt and Siegel 1978; Doyle et al. 1977; Feldman 1995; Mooney et al. 1985; Nilsson 1994; Siegel and Doyle 1975a, b; Siegel et al. 1977, 1992; Siegel and Mooney 1987). The pathogenesis of stress-induced bone mineral changes is an activation of the hypophyseal-adrenal/thyroid axis, leading to enhanced parathyroid and cortisol hormone secretion and increased bone resorption, while bone formation is decreased (Colborn et al. 1993, Damstra et al. 2002; Feldman 1995; Ganong 1991, Selye 1973). Other hypotheses on disruption of bone mineral status include altered mitotic rates, changes in local subcellular calcium transport or decreased protein synthesis (Siegel and Mooney 1987).

Organochlorines like PCBs (PolyChlorinatedBiphenyls), DDTs (DichloroDiphenylTrichloroethanes), CHLs (CHLordanes), HCHs (HexaCycloHexanes), dieldrin, HCB (HexaChloroBenzene), PBDEs (PolyBrominatedDiphenylEthers) and aryl hydrocarbon receptor (AhR) active organochlorines (*e.g.* polychlorinated dibenzo-*p*-dioxins, dibenzofurans and non-*ortho* chlorine-substituted PCBs) are all lipophilic (low degradable) chemicals, pesticides, or unwanted chemical by-products (*e.g.* de March et al. 1998). In general, the presence of such compounds in the arctic marine environment is the result of long-range atmospheric transport from lower latitude sources in more industrial areas of the world, where outputs and use of, *e.g.*, PCB peaked in the 1960's (de March et al. 1998). Due to their lipophilicity and chemical properties, organochlorines and PBDEs persist in the environment (AMAP 2004; Colborn et al. 1993; Damstra et al. 2002; de March et al. 1998). In polar bears, organochlorines are consequently transferred transplacentally from mother to fetus and via lactation, resulting in fetal and neonatal exposures that have the potential for adverse health effects, *e.g.* on growth and development (Bernhoft et al. 1997; Birnbaum 1994; Polischuk et al. 1995, 2002).

DDTs and PCBs in humans have been associated with low bone mineral density (Alveblom et al. 2003; Beard and Young 2000; Glynn et al. 2000) through their action as exogenous agonists and antagonists to naturally endogenous hormones (Damstra et al. 2002). Various organochlorines have also been linked to periodontitis and osteoporosis in marine fish and mammal wildlife (Bengtsson et al. 1985, Bergman et al. 1992, de Guise et al. 1995; Lind et al. 2003; Lind et al. 2004; Mortensen et al. 1992; Schandorff 1997a; Zakharov and Yablokov 1990) and in the laboratory (Fernie et al. 2003; Jamsa et al. 2001; Lind et al. 1999; Lind et al. 2000a, 2000b; Render et al. 2000a, 2000b, 2001; Singh et al. 2000; Valentine and Soulé 1973). In various mamma-

lian wildlife, osteopenia and macroscopic pathology have been examined in bone during distinct periods of exposure to anthropogenic pollutants have been examined in *e.g.* grey seal (*Halichoerus grypus*), ringed seal (*Phoca hispida*) harbour seal (*Phoca vitulina*) and alligator (*Alligator mississippiensis*) (Bergman et al. 1992; Lind et al. 2003; Lind et al. 2004; Mortensen et al. 1992; Zakharov and Yablokov 1990; Schandorff 1997a, 1997b; Sonne-Hansen et al. 2002). The studies showed relationhips between organochlorines and exostosis, periodontitis, loss of alveolar bone structures, osteoporosis, widening of the canine opening and enlargement of the foramen mentalia.

Polar bears (*Ursus maritimus*) from East Greenland, Svalbard and the Kara Sea carry higher loads of organochlorines than polar bears elsewhere in the Arctic due to their reliance on blubber from ringed seal (*P. hispida*) and bearded seal (*Erignathus barbatus*) (*e.g.* AMAP 2004; de March et al. 1998; Lie et al. 2003; Norstrom et al. 1998). Recent studies of polar bears from Svalbard have indicated that high levels of organochlorines are negatively associated with retinol (vitamin A) and levels of thyroid hormones (Skaare et al. 2001) and possibly also negatively affect sex steroids and reproductive organs (female pseudohermaphrodites) - although these latter mechanisms are not clearly understood (Haave et al. 2003; Oskam et al. 2003; Sonne et al. in press; Wiig et al. 1998). Other studies have associated high levels of organochlorines with low levels of IgG suggesting a possible immunotoxic effects on the IgG levels (Bernhoft et al. 2000; Lie et al. 2004, submitted). Overall, these studies support the notion that organochlorines may cause disruption and thereby potentially affect bone mineral composition.

To determine whether exposure to organochlorines and PBDEs may have adversedly affected bone mineral composition in polar bears, we compared BMD in skulls of 41 individual polar bears collected in East Greenland during the supposed pre-polluted period (1892-1960) with 98 polar bear skulls collected during the supposed polluted period (1961-2002). The year 1961 was chosen as dividing year due to the transport of organochlorines (and later PBDEs) from lower latitudes to East Greenland (Norstrom et al. 1998; de March et al. 1998, AMAP 2004). Furthermore, we examined a sub-set of 58 of the individuals collected during the pollution period to determine if BMD was related to body burden of various organochlorines and PBDEs.

## Materials and methods

### Sampling and age estimation

A sample of 139 East Greenland polar bear skulls (sampled between Skjoldungen at 63º15'N and Danmarks Havn at 76º30'N) sampled during 1892-2002 was studied. The age determination was carried out by counting the cementum Growth Layer Groups (GLG) of the lower $I_3$ after decalcification, thin sectioning (14µm) and staining (toluidine blue) using the method described by *e.g.* Hensel and Sorensen (1980) and Dietz et al. (1991). For analyses, the individuals were then categorised into subadults, adult males and adult females by these criteria: adult males ≥ 6 years, adult females ≥ 5 years and others as subadults (*e.g.* Rosing-Asvid et al. 2002). Regarding skull samples from 1892-1987 the sex was available from the expedition files, and in case of absence of this information ($n$=9) the determination was based on skull morphology.

## Osteodensitometry

X-ray osteodensitometry was applied to detect osteopenia (osteoporosis) by use of a Norland XR 26 X-ray bone densitometer (Norland Corporation, Wisconsin, USA) which determined the bone mineral density (calcium-phosphate; hydroxyapatite) during a dual X-ray absorptiometry (DXA). The skulls were scanned in "*Research*" mode (speed: 60 mm / sec; resolution: 3.0 x 3.0 mm; width: 24,9 cm) and analysed in XR software revision 2.4®, which generated a picture of the bone segment and calculated the bone mineral density of hydroxyapatite (BMD; g $cm^{-2}$) (*Ibid.*) (Fig. 1).

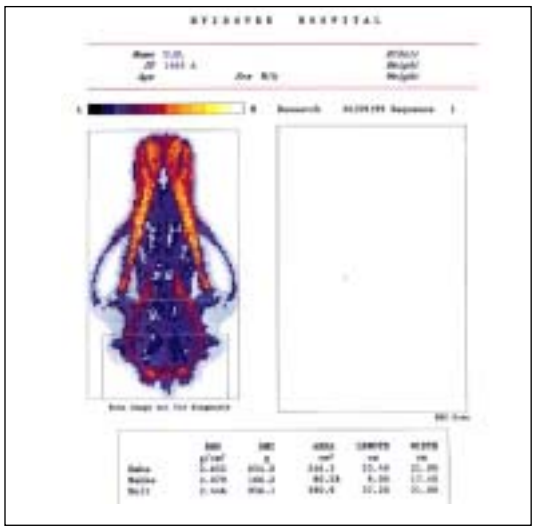

**Figure 1**
DXA scanning image of a 12-year-old female East Greenland polar bear sampled in 1972. Note the high density areas of cortical bone tissue (light) and the lower density areas of trabecular bone tissue (dark).

To ensure that BMD in the skull represents the mineral status of the skeletal system in general, a study was conducted where the BMD of the skull, one femur and three lumbar vertebrae were compared in a sub-set of 13 polar bears (3 subadults, 2 adult females and 8 adult males) from the Copenhagen Zoo and East Greenland. The DXA-scanner was daily calibrated using a phantom with known mineral density. In addition the precision was tested by a 10 time rescanning (mean=521.96 g $cm^{-2}$, SD=0.60) which from the formula [1 – (SD / mean) x 100%] gives a precision of 99.88%. Fragmentation and loss of teeth material caused by handling and lead shot was thought to be a problem. A double determination of the BMD in 2 skulls (#5483 and #2891) with and without incisors, canines, premolars and molars showed that loss of half or more of the material of the large canines altered the result significantly. As none of the canines in the entire material were fragmented to such a degree, fragmentations were not considered a problem.

## Contaminant analyses

Polar bear subcutaneous adipose tissue samples (*n*=58) were analysed for PCBs DDTs, HCHs, CHLs, HCB, dieldrin and PBDEs as described elsewhere (Dietz et al. 2004; Luross et al. 2002; Sandala et al. 2004). Sum (Σ) PCBs are the total concentrations of the 51 individual or co-eluting congeners (if detected): CB # 31 / 28, 52, 49, 44, 42, 64 / 71, 74, 70, 66 / 95, 60, 101 / 84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129 / 178, 182 / 187, 183, 128, 174, 177, 171 / 202 / 156, 200, 172, 180, 170 / 190, 201, 203 / 196, 195, 194, 206. Σ-DDTs are the sum of *4,4'*-DDT, *4,4'*-DDD and *4,4'*-DDE. Σ-HCHs are

the sum of the α-, β- and γ-hexachlorocyclohexane. ΣCHLs are the total concentrations of oxychlordane, *trans*-chlordane, nonachlor III (MC6), *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. Σ-PBDE concentrations are the total of 35 individual or co-eluting congeners (if detected): BDE# 10, 7, 11, 8, 12/13, 15, 30, 32, 28/33, 35, 37, 75, 71, 66, 47, 49, 77, 100, 119, 99, 116, 85, 155/126, 105, 154, 153, 140, 138, 166, 183, 181, 190 (Muir et al. in prep.). All contaminant data is given in ng/g lipid weight (l.w.).

### Statistics

The BMD showed no deviation from normality (Shapiro-Wilk test) while contaminant data was log-transformed (base e) prior to the analyses in order to meet the criteria of normality and homogeneity of the variance. The significance level was set to $p \leq 0.05$, while significance levels of $0.05<p \leq 0.10$ was considered a trend. *First*, the condylobasal skull-length versus age was tested within each group (*i.e.* subadults of both sexes, adult females and adult males) in an analyses of covariance with skull-length as dependent variable, periods (before and after 1960 respectively) as class variables, age as covariable and their 1st order interaction links (age*period). The result from this analysis showed that the skull-length vs. age relation was the same in the two periods which justified the use of non-length-corrected skull BMD in the further analyses (all: $p>0.26$). *Secondly*, the BMD versus age relationship was tested by a linear regression analyses (BMD as dependent variable and age as independent variable) for subadults of both sexes, adult females and adult males. To test for *period differences*, an ancova was used with BMD as dependent variable, age/sex (subadult females, subadult males, adult females and adult males) and period (before and after 1960 respectively) as class variables, the age as covariable and the 1st order interactions links (age*period, age*age/sex and age/sex*period) between these variables. The model was successively reduced for non-significant interactions ($p>0.05$) judged from the type-III sum of squares, and the significance of the remaining factors was evaluated from the final model (LSMean). A *temporal trend* over the entire period 1892-2002 was analysed by a multiple regression analysis with skull BMD as the dependent variable and the individual age and year of kill as explanatory variables for subadults of both sexes, adult females and adult males respectively (the relationship was evaluated from the parameter estimate, $r^2$ and *p*-value). The *relation between age/sex groups and contaminants* was analysed within a one-way anova on the log-transformed contaminant data and significant results were tested among each other by Tukey's *post hoc* test. The *Skull BMD versus contaminants* (∑-PCBs, ∑-DDTs, ∑-CHLs, HCB, ∑-HCHs, dieldrin and ∑-PBDEs) relationships were explored by multiple regressions with skull BMD as the dependent variable and the age and contaminant concentrations as explanatory variables within age/sex groups (subadults of both sexes, adult females and adult males). Finally the relationship between levels of contaminants and BMD was evaluated from the parameter estimate, $r^2$ and *p*-value.

## Results

We found a highly significant correlation between BMD (bone mineral density) in skull and femur ($r=0.99$; $p<0.001$; $n=13$), and skull and vertebrae ($r=0.97$; $p<0.001$; $n=8$). These results justified the use of BMD measurements in skull to reflect the status of the skeletal system although information on body conditions and nutritional stressors, relevant for osteoblastic and -clastic activity, was not available.

## Skull BMD and age/sex differences

BMD was analysed in 139 skulls representing the period from 1892 to 2002 and consisted of 64 subadults, 40 adult females and 35 adult males. The BMD increased with age in subadults ($p<0.001$) but not adults (both: $p>0.05$) (Fig. 2). BMD differed between males and females ($p<0.01$) in the order: subadult females<subadult males<adult females<adult males. Furthermore, BMD in females 14-23 years of age seemed to decline significantly with age ($p<0.04$).

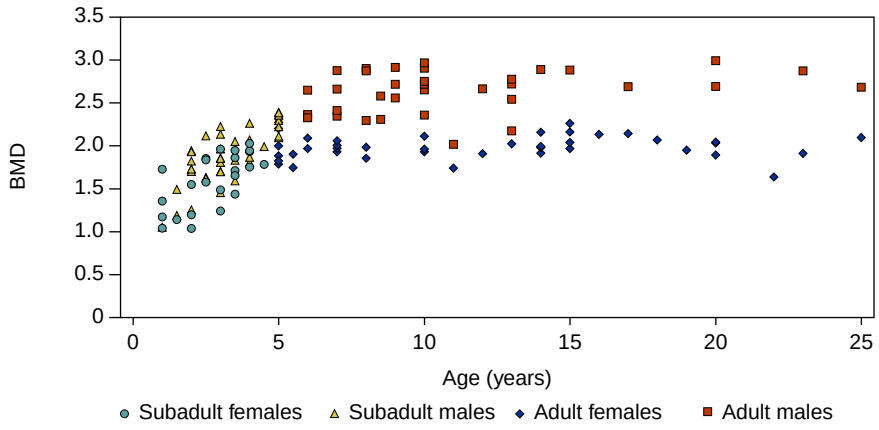


**Figure 2**
BMD (g cm$^2$) in skulls from East Greenland polar bears versus individual age (years).

## Period differences and temporal trends in skull BMD

Forty-one skulls were available from the supposed pre-pollution period (1892-1960) and 98 from the supposed pollution period (1961-2002) (Table 1a). BMD in skulls sampled in the pollution period was significantly lower than BMD sampled in skulls from the pre-pollution period for subadults and adult males ($p<0.05$), but not for adult females ($p>0.9$) (Table 1a). In addition, the multiple regression analyses of BMD versus individual age and year of kill (1892-2002) showed that BMD decreased over the entire period in adult males ($p<0.01$) and a similar trend was found for subadults ($p=0.07$) (Table 1b). There was no BMD time trend for adult females ($p>0.5$).

**Table 1a**
Skull bone mineral density (BMD) in subadult and adult East Greenland polar bears from 1892 to 2002.

| Periode | Variable | Subadult females* | | Subadult males* | | Adult females* | | Adult males** | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n |
| | BMD | 1.67 ± 0.37 | 7 | 2.22 ± 0.19 | 5 | 1.99 ± 0.13 | 9 | 2.73 ± 0.21 | 20 |
| 1892-1959 (periode 1) | Age | 2.6 ± 1.3 | 7 | 4.4 ± 1.3 | 5 | 12.7 ± 3.7 | 9 | 11.5 ± 4.5 | 20 |
| | BMD | 1.55 ± 0.3 | 17 | 1.85 ± 0.32 | 35 | 1.98 ± 0.13 | 31 | 2.49 ± 0.24 | 15 |
| 1960-2002 (period 2) | Age | 2.8 ± 1 | 17 | 3.2 ± 1.1 | 35 | 12.1 ± 6.3 | 31 | 10.7 ± 5.5 | 15 |

Data are divided on period 1 (1892-1960: supposed organochlorines and PBDEs *non*-polluted) and period 2 (1961-2002: supposed organochlorines and PBDEs polluted). BMD (g cm$^{-2}$) was obtained by DXA-skanning of the entire skull and age (years) was obtained by counting the GLG of the lower $I_3$ tooth. Data is given in mean±SD and number of observations is given in brackets. *: BMD in period 2 significant lower compared to period 1 for the given age/sex group at the p≤0.05 level. **: BMD in period 2 significant lower compared to period 1 for the given age/sex group at the p≤0.01 level.

**Table 1b**
Significant results from the multiple regression analyses of skull bone mineral density (BMD) versus age and year of kill in East Greenland polar bears during 1892-2002.

| Age/sex group | Equation | $r^2$ | $p_{age}$ | $p_{yok}$ | n |
|---|---|---|---|---|---|
| Subadults | BMD=0.193*age–0.00254*yok+6.3 | 0.64 | <0.001 | 0.07* | 40 |
| Adult males | BMD=0.014*age–0.00324*yok+8.8 | 0.31 | 0.2 | <0.01** | 35 |

The equation is given as: [BMD=A*age+B* yok+C]. Dependent variable: BMD (g $cm^{-2}$). Explanatory variables: Age (years) and year of kill (yok; 1892-2002). A, B and C: specific parameter estimates. $r^2$: regression coefficient of the model, $p_{age}$: p-value for age and $p_{yok:}$ p-value for year of kill. *: *i.e.* non-significant trend of BMD decline over the entire period 1892-2002 at the 0.05<p≤0.10 level. **: *i.e.* significant BMD decline over the entire period 1892-2002 at the p≤0.01 level.

## Skull BMD and contaminants

The range and variation of organochlorine and PBDE contaminants (ng/g l.w.) in the 58 polar bears sampled during 1999-2002 are presented in Table 2. Levels of ∑-DDTs, dieldrin, ∑-HCHs and ∑-PBDEs was not different between subadults, adult females and adult males (all: $p$>0.07). But, levels of ∑-PCBs were higher in adult males when compared to adult females ($p \leq 0.05$). Further information on the relation between organochlorines and age, sex and season in East Greenland polar bears from 1999-2002 is available in Dietz et al. (2004) and Sandala et al. (2004).

**Table 2**
Concentrations of various contaminants in subcutaneous adipose tissue of 58 East Greenland polar bears sampled during 1999-2001.

| Compound | Subadults (n=35) | Adult females (n=14) | Adult males (n=9) |
|---|---|---|---|
| ∑-PCBs | 6597±2726 (6089) | 5334±2150* (5770) | 8637±4111* (8280) |
| ∑-CHLs | 1598±884 (1469) | 1379±591 (1353) | 1055±517 (914) |
| ∑-DDTs | 392±209 (376) | 358±149 (366) | 481±331 (496) |
| ∑-HCHs | 196±68 (172) | 195±186 (151) | 294±210 (181) |
| Dieldrin | 210±100 (196) | 174±70 (154) | 177±81 (172) |
| HCB | 99±84 (70) | 75±82 (51) | 51±28 (48) |
| ∑-PBDEs | 62±33 (53) | 53±17 (53) | 52±16 (49) |

Contaminant data is given in ng/g l.w. within groups of subadults of both sexes, adult females and adult males. Data is given in mean±SD and the median in brackets. *: significant difference between adult females and males at the p≤0.05 level.

BMD was found to be negatively correlated with levels of ∑-PCBs ($p$<0.04) and ∑-CHLs in subadults ($p$<0.03) while BMD was negatively correlated to ∑-DDTs ($p$<0.02) and dieldrin ($p$<0.002) in adult males (Table 3). In addition, a trend of ∑-PBDEs being negatively correlated to BMD in subadults was found ($p$=0.06) while no significant relations were found for adult females (Table 3).

**Table 3**
Significant results from the multiple regression analyses of skull bone mineral density (BMD) versus age and contaminant concentrations in East Greenland polar bears sampled during 1999-2001.

| Age/sex group | Equation | $r^2$ | $p_{age}$ | $p_{cont}$ | n |
|---|---|---|---|---|---|
| Subadults | BMD=0.26*age–0.25*[Ln(∑-PCBs)]+3.1 | 0.59 | <0.001 | <0.04** | 35 |
| Subadults | BMD=0.24*age–0.19*[Ln(∑-CHLs)]+2.4 | 0.6 | <0.001 | <0.03** | 35 |
| Subadults | BMD=0.25*age–0.18*[Ln(∑-PBDEs)]+1.69 | 0.58 | <0.001 | 0.06* | 35 |
| Adult males | BMD=0.02*age–0.17*[Ln(∑-DDTs)]+3.4 | 0.69 | >0.08 | <0.02** | 9 |
| Adult males | BMD=-0.005*age–0.37*[Ln(Dieldrin)]+4.5 | 0.85 | 0.43 | <0.002*** | 9 |

The equation is given as: [BMD=A*age+B*Ln(cont)+C]. Dependent variable: BMD (g $cm^{-2}$). Explanatory variables: Age (years) and Ln-transformed contaminant concentration [Ln(ng/g l.w.) ]. A, B and C: specific parameter estimates. $r^2$: regression coefficient of the model, $p_{age}$: p-value for age and $p_{cont}$: p-value for contaminants. *: *i.e.* non-significant trend of a negative correlation between BMD and Ln(∑PBDEs) at the 0.05<p≤0.10 level. **: *i.e.* significant negative correlation between BMD and organochlorine contaminant group at the p≤0.05 level; ***: *i.e.* significant negative correlation between BMD and organochlorine contaminant group at the p≤0.01 level.

## Discussion

### BMD and age/sex differences

The high correlation between skull BMD and femur and vertebrae, respectively, is usefull as skull samples of polar bears (and other mammals) are present at national zoological museums all over the world, which make various time-trend bone studies possible. Our results clearly showed that skull BMD increased more rapidly in subadults compared to adults in accordance with previous studies of ringed seals from NW Greenland (Sonne-Hansen et al. 2002). Female polar bears usually give birth to two cubs every third year (December) and mobilize and tranfer large amounts of calcium and phosphate during the gestation and *post partum* (suckling) period lasting for up to two years (Ramsay and Stirling 1988). In this period calcium is used for foetal skeletal production and maintenance of the mothers and her offspring's calcium-phosphate homeostasis (*Ibid.*). As the female polar bear mobilizes these large amounts of calcium and phosphate, it could be expected that adult females have a lower BMD compared to adults males. Such a difference was also found in the present study. Similiar differences have been found in humans (*e.g.* Van Langendonck et al. 2002). As suggested for humans, the marked difference in BMD between the sexes could be the result of a higher muscle mass and strength in males, leading to higher biomechanical loading of the bone. This would lead to an increased bone formation through the stimulation of mechanotransduction system in the osteocytes (*Ibid.*).

Earlier studies show that sufficient levels of sex steroids (estrogens, androgens) are important in the development of the human cortical bone structures in boys, girls, teenagers, adults and elderly (Hampson et al. 2002; Juul 2001; Leder et al. 2001; Szulc et al. 2001). On the other hand, high levels of estrogen active substances (intrinsic, extrinsic) stimulate the expression of secondary sexual characteristics (*Ibid.*). Therefore growth delay and osteopenia (osteoporosis) have been associated with hypogonadism and lower estrogen levels in both sexes (Leder et al. 2001; Nelson 2003; Szulc et al. 2001). Indications of such age-related decrease in BMD in females was found in the present study probably associated with a menopause phase after the 15th year of age but this requires a larger sample size (Fig. 2) (Derocher and Stirling 1994).

### Period differences and temporal trends in skull BMD

In both analyses of subadults of both sexes and adult males the individuals from the pre-pollution period had a higher skull BMD compared to the polluted period. These results suggest that there is a linkage between decreased BMD for bears from the polluted period, and exposure to environmental stressors compared to bears in the pre-pollution period. Two major environmental stressors could be linked to mineral loss in polar bear skulls: anthropogenic organochlorine compounds and PBDEs and / or climate oscillations (AMAP 2004; de March et al. 1998; Førland et al. 2002). Concentrations of *e.g.* ∑-PCBs in the adipose tissue of East Greenland polar bears have over the last four decades reached levels that can elicit adverse biological effects on immunological parameters and vitamin A (stress), which may be linked to the present decrease in skull BMD (AMAP 2004; de March et al. 1998). However, during the same period global warming has resulted in a reduction in the ice coverage in the East Greenland area (Comiso 2002; Rothcock et al. 1999). Although population ecology has not been studied in East Greenland, the situation is probably similar for polar bears from the Hudson Bay area in Canada (Stirling et al. 1999). A reduction of the sea ice in the Hudson Bay area has reduced the bears' access to ringed seals resulting in reduced body condition and lowered natality in the polar bears (*Ibid.*).

Temporal differences with respect to potential effects of PCB and DDT exposure on periodontitis and osteoporosis in grey seal and harbour seal was investigated by Bergman et al. (1992); Mortensen et al. (1992) and Schandorff (1997). They found exostosis and periodontitis often with substantial loss of alveolar bone in mandible and maxilla (osteoporosis). These changes could have been caused by hormonal imbalance potentially induced by PCBs and DDTs with malformation of the calcium helix structures around the collagen matrix (DeLillis 1989). These results are further supported by the investigations of Render et al. (2000a, b, 2001). However it must be noted that the range in Σ-PCB and Σ-DDT levels in the seals were orders of magnitude higher compared to levels in the present polar bears (Blomkvist et al. 1992).

Lind et al. (2003) investigated the bone mineral density (g $cm^{-3}$) in the male grey seals ($n$=43) reported above by Bergman et al. (1992). The method used was pQCT (*p*eripheral *q*uantitative *c*omputed *t*omography) which made it possible to distinguish between cortical and trabecular bone in *os mandibularis* and *os radius* respectively (DXA-scanning used in the present study gives the average of trabecular and cortical bone density). Three sample groups of seals were compared: 1850-1955 (no pollution); 1965-1985 (high pollution) and 1986-1997 (fairly low pollution). They found that radius trabecular bone mineral density was significantly higher in the fairly low pollution period (1986-1997) compared to the high pollution period (1965-1985) while for mandible cortical bone mineral density was significantly lower in the fairly low pollution period (1986-1997) compared to the no pollution period (1850-1955). Our study of BMD in East Greenland polar bears supports the findings of Lind et al. (2003).

### BMD levels and contaminants

Bone density expresses the bone mineral composition determined by the activity of osteoblastic bone formation and osteoclastic bone resorption which is regulated by androgens and estrogens through cytokines (Manalagas and Jilka 1995; Manalagas et al. 1995). Studies on Svalbard have shown that PCB may negatively influence plasma testosterone levels (Oskam et al.

2003) and plasma retinol and thyroid hormone levels in polar bears (Skaare et al. 2001). These studies all indicate that organochlorines in Svalbard polar bears (and likely also East Greenland bears, as the OHC levels are comparable) potentially affects the endocrine homeostasis, which again may lead to bone mineral loss (osteoporosis) (*Ibid.*). Another polar bear study from Svalbard associated high levels of organochlorines with low levels of IgG suggesting possible immunotoxic effects (Bernhoft et al. 2000, Lie et al. 2004, submitted). This potential effect may lower the immune response and enhance stress with increased cortisol levels), which potentially affects the bone mineral composition (osteoporosis).

The present study indicated that high concentrations of Σ-PCBs and Σ-CHLs are associated with reduced skull BMD in subadults and that Σ-DDTs and dieldrin are associated with reduced skull BMD in adult males. These BMD relationships with Σ-PCBs, Σ-DDTs, Σ-CHLs and dieldrin concentrations in adult males and subadults of both sexes may suggest endocrine-related effects (*e.g.* AMAP 2002; Birnbaum 1994; Damstra et al. 2002; de March et al. 1998; Lind et al. 2003; Lind et al. 2004). For example, PCBs and DDTs have shown in vitro and vivo to be weak agonists antagonists of estrogen receptor-mediated activity, or OC-mediated induction of CYP450 activity can impact circulating sex hormone levels (*e.g.* estrogens) (Navas and Segner 1998) and this is also of relevance in the polar bear (*Ursus maritimus*) (*e.g.* Letcher et al. 1996). Relationships between 4,4'-DDE concentrations and BMD in humans have been reported (Beard and Jong 2000; Glynn et al. 2000). Glynn et al. (2000) found significant negative correlations between 4,4'-DDE and BMD in 68 sedentary women (where concentrations are lower compared to the present polar bears), and concluded that 4,4'-DDE may also have a negative effect on BMD in men (with contaminant levels comparable to those found in the polar bears). Lind et al. (2004) investigated the relationship between DDTs and bone composition in juvenile female american alligators (*Alligator mississippiensis*) in Lake Apopka. Compared to a non-polluted reference alligator subpopulation the tibial trabecular BMD was increased and the authors suggested that environmental estrogenic copounds (DDTs and its metabolites a.o.) disrupted the normal bone remodelling process (inhibition of osteoclast activity) which had resulted in increased BMD.

Guo et al. (1994) found that for primiparous PCB contaminated mothers (Yu-Cheng; rice-oil disease) their children (n=25) were significantly smaller and had less total lean mass, less soft tissue mass but not lower bone mineral density compared to a control group. The PCB levels in the children (serum) were 10.3 ng/g l.w. and were lower than the levels in the present study. Alveblom et al. (2003) investigated the incidence of osteoporotic fractures in fishermen and their wives from the Baltic Sea (high pollution) and compared these to fishermen from the west coast of Sweden (low pollution) as controls. For vertebral fractures there was a significantly higher IRR (incidence rate ratio) for east coast (Baltic) women compared to west coast women and a similiar but non-significant tendency was found for men. The PCB concentration (10 congeners) was 2000 ng/g l.w. (serum) which was significantly higher compared to the west coast population but lower compared to the range in the subcutaneous adipose tissue of East Greenland polar bears. These environmental studies support the findings of negative associations between PCBs/DDTs and BMD levels in East Greenland polar bears.

A negative correlation was observed in the present bears between ΣPBDE concentrations in adipose tissue and BMD in subadults. Disturbances in thyroid function and developmental toxicity (histopathology) have been

shown to be associated with PBDEs in laboratory rats (*e.g.* de Wit 2002) as well as polar bears from Svalbard (Skaare et al. 2001).

## Conclusions

Skull bone mineral density (BMD) increased with age in subadults and was higher in males than in females at all ages. For adult females older than 13 years of age a menopausal BMD decrease was indicated but a further examination requires a larger sample size. BMD in skulls from subadult females, subadult males and adult males sampled in the supposed pollution period (1961-2002) was significantly lower than BMD in skulls from the supposed pre-pollution period of organochlorine and PBDE compunds (1892-1960). Furthermore, correlative relationships suggested that Σ-PCB, Σ-CHL, dieldrin and Σ-DDT exposure negatively influenced BMD in skulls from subadults of both sexes and adult males.

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# Paper III-b
# Periodontitis and tooth wear in East Greenland polar bears (*Ursus maritimus*) during 1892-2002

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## Abstract

The anatomy of 272 East Greenland polar bear skulls and mandibles sampled in the period 1892-2002 were examinated in relation to the relatively high adipose concentrations of organohalogens found in this bear population since 1976. The dominating macroscopic pathological change was periodontitis and in the most severe cases with a substantial loss of alveolar bone structure accompanied tooth wear. There was a significant increase in the prevalence of periodontitis with age ($p<0.01$; $X^2$ test) but no difference between adult females and adult males ($p>0.2$). We could not find a difference in the prevalence of periodontitis between period of no pollution (1892-1960) and with pollution (1961-2002) ($p>0.7$; $X^2$-test) like we could not found a relation to individual adipose concentrations of organohalogens in 79 individuals. We propose that tooth wear in free-ranging polar bears may be a mortality factor.

*Key words:* polar bear, *Ursus maritimus*, PCBs, DDTs, CHLs, dieldrin, PBDEs, periodontitis, tooth wear.

## Introduction

Organohalogens like PCBs (PolyChlorinatedBiphenyls), DDTs (DichloroDiphenylTrichloroethanes), CHLs (CHLordanes), HCHs (HexaCycloHexanes), dieldrin, HCB (HexaChloroBenzene), PBDEs (PolyBrominatedDiphenylEthers), and aryl hydrocarbon receptor (AhR) active contaminants including polychlorinated dibenzo-*p*-dioxins, dibenzofurans and non-*ortho* chlorine-substituted PCBs (i.e., CB-77, CB-126 and CB-169) are all lipophilic (low degradable) chemicals, pesticides, or unwanted chemical by-products (*e.g.* de March *et al.* 1998). In general, the presence compounds in the Arctic marine environment is the result of long-range atmospheric transport from lower latitude sources in more industrial areas of the world, where outputs and use of, *e.g.*, PCB peaked in the 1960's (de March *et al.* 1998). Due to their lipophilicity; organohalogens persists in the environment (Colborn *et al.* 1993, de March *et al.* 1998, Damstra *et al.* 2002, AMAP 2004). In mammals, organohalogens are consequently transferred transplacentally from mother to foetus and via lactation, resulting in foetale and neonatale exposures that

have the potential for adverse health effects, *e.g.* on growth and development (Takagi *et al.* 1976, Tanabe *et al.* 1982, Koppe *et al.* 1992, Birnbaum 1994, Polischuk *et al.* 1995, Bernhoft *et al.* 1997, Polischuk *et al.* 2002).

Various organohalogens have been linked to dysosteogenesis (periodontose) in mammal wildlife (Zakharov and Yablokov 1990, Bergman *et al.* 1992, Mortensen *et al.* 1992, Schandorff 1997a) and in the laboratory (Render *et al.* 2000a,b, Render *et al.* 2001). In various mammalian wildlife, periodontitis in skull and mandibles during distinct periods of exposure to anthropogenic pollutants have been examined in *e.g.* grey seal (*Halichoerus grypus*), ringed seal (*Phoca hispida*) and harbour seal (*Phoca vitulina*) (Zakharov and Yablokov 1990, Bergman *et al.* 1992, Mortensen *et al.* 1992, Schandorff 1997a-b). These studies showed relationhips between organohalogens and exostosis, periodontitis, loss of alveolar bone structures, osteoporosis, widening of the canine opening and enlargement of the foramen mentalia.

Polar bears (*Ursus maritimus*) from East Greenland, Svalbard and the Kara Sea carry higher loads of organohalogens than polar bears elsewhere in the Arctic due to their reliance on blubber from ringed seal (*P. hispida*) and bearded seal (*Erignathus barbatus*) (*e.g.* Bernhoft *et al.* 1997; Dietz *et al.* 2004, de March *et al.* 1998, Norstrom *et al.* 1988, 1998; Andersen *et al.* 2001, Derocher *et al.* 2002, Lie *et al.* 2003, AMAP 2004). Recent studies of polar bears from Svalbard have indicated that high levels of organohalogens negatively affect retinol (vitamin A) and thyroid levels (Skaare *et al.* 2001) and possibly also negatively affect on sex steroids and reproductive organs (female pseudohermaphrodites) - although these latter mechanisms are not clearly understood (Wiig *et al.* 1998, Haave *et al.* 2003, Oskam *et al.* 2003, Sonne *et al.* In press). Other studies have associated high levels of organohalogens with low levels of IgG suggesting a possible immunotoxic effects on the IgG answer (Bernhoft *et al.* 2000, Lie *et al.* 2004, Lie *et al.* submitted). Overall, these studies support the notion that organohalogens may cause endocrine disruption in the polar bear and thereby potentially lower the immune response.

To determine whether exposure to organohalogens may have contributed to periodontitis and alveolar bone structure, 272 East Greenland polar skulls collected from 1892 to 2002 were compared to detect a time trend in the prevalence between the period without pollution (1892-1960) and with pollution (1961-2002). Finally, the prevalence of periodontitis in a subset of 79 individuals was analysed in relation to individual adipose body burden of various organohalogens.

## Materials and methods

### Sampling and preparation

A sample of 272 skulls and 52 bacula from East Greenland (sampled between Skjoldungen at 63º15'N and Danmarks Havn at 76º30'N) was studied. The specimens had been sampled by scientific expeditions ($n$=167) from 1892 to 1987 and stored at the Zoological Museum in Copenhagen (Denmark) and during our recent collection ($n$=105) with the assistance of local hunters in Scoresby Sound (ca. 71 ºN), 1999 to 2002. The preparation method of the "historical" specimens and the recently sampled skulls and bacula were macerated and boiled so that muscles and tendons could be removed gently prior to $H_2O_2$ oxidation for 24 to 48 hours. Samples of subcutaneous adipose tissues from the 105 newly sampled bears were shipped frozen from

Scoresby Sound to Copenhagen, where they were transferred to cleaned storage glasses with aluminum sealed lids and further storage at -20 ºC. This is described in details in Dietz *et al.* (2004).

### Age estimation

The age determination was carried out by counting the cementum Growth Layer Groups (GLG) of the lower $I_3$ after decalcification, thin sectioning (14µm) and staining (toluidine blue) using the method described by *e.g.* Hensel and Sorensen (1980) and Dietz *et al.* (1991).

Regarding skulls samples from 1892-1987 the sex was available from the expedition files, and in case of absence of this information (*n*=9) the determination was based on skull morphology. For analyses, the individuals were then categorised into subadults, adult males and adult females by these criteria: adult males ≥ 6 years, adult females ≥ 5 years and others as subadults (*e.g.* Rosing-Asvid *et al.* 2002).

### Periodontitis and tooth wear

A macroscopic pathological examination focusing on periodontitis and alveolar bone loss was applied to all 272 skulls. In addition to severe bone loss, tooth wear was noted.

### Contaminant analyses

#### PCBs and OCs

Polar bear adipose tissue samples (*n*=79) were analysed for PCBs, CHLs, DDTs, dieldrin, HCHs, HCB and PBDEs according to Sandala *et al.* (2004) and Dietz *et al.* (2004) at the Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada. An external standard quantification approach used for PCBs and OCs in the adipose tissues was based on peak area of the GC-µECD response, which is described in detail in Dietz *et al.* (2004). Briefly, ∑-PCBs is the sum (s) of the concentrations of the 51 individual or co-eluting congeners (if detected): CB # 31 / 28, 52, 49, 44, 42, 64 / 71, 74, 70, 66 / 95, 60, 101 / 84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129 / 178, 182 / 187, 183, 128, 174, 177, 171 / 202 / 156, 200, 172, 180, 170 / 190, 201, 203 / 196, 195, 194, 206. ∑DDTs is the sum of *4,4'*-DDT, *4,4'*-DDD and *4,4'*-DDE. ∑-HCHs is the sum of the α-, β- and γ-hexachlorocyclohexane. ∑-CHLs is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. Contaminant fractions were subsequently sent to the National Water Research Institute (Environment Canada, Burlington, Ontario, Canada L7R 4A6 (NWRI)) for determination of brominated diphenyl ether (PBDE) flame retardants.

#### PBDEs

BDPEs (*n*=79) were determined by electron capture negative ion (low resolution) MS using an external standard. Briefly, ∑-PBDEs is the sum (s) of the concentrations of the 35 individual or co-eluting congeners (if detected): BDE# 10, 7, 11, 8, 12 / 13, 15, 30, 32, 28 / 33, 35, 37, 75, 71, 66, 47, 49, 77, 100, 119, 99, 116, 85, 155 / 126, 105, 154, 153, 140, 138, 166, 183, 181, 190. Gas chromatographic conditions for the PBDEs were described by Luross *et al.* (2002) and Muir *et al.* (in preparation).

### Dioxin-like contaminants

Extraction and analyses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and non-*ortho* PCB congener 3,3',4,4',5-pentachlorobiphenyl (CB-126) in polar bear fat was conducted by Axys Analytical Services (Sidney, BC, Canada) used previously described techniques with only minor modification (U.S. EPA, 1998, 1999). 2,3,7,8-TCDD and CB-126 were chosen as model dioxin-like contaminants since the former is the most potent AhR agonist among all dioxin and furan congeners, and CB-126 is the most potent AhR agonist among all PCB congeners. Briefly, fat samples were homogenized with $Na_2SO_4$ and spiked with mixture of $^{13}$C-labelled non-*ortho* PCBs and 2,3,7,8-TCDD surrogate standards to monitor extraction efficiency and to quantify these analytes in the samples using an isotope dilution approach. Samples were extracted in toluene via Soxhlet and concentrated. Extracts were cleaned-up and PCDD/Fs were isolated through a series of chromatographic columns (U.S. EPA 1998). Analyses was performed using a high-resolution mass spectrometer coupled to a high-resolution gas chromatograph (HRGC-HRMS) equipped with a DB5 capillary chromatography column (60 m ´ 0.25 mm i.d. ´ 0.1 µm film thickness; J&W Scientific, Folsom, CA, USA). All analytical procedures were carried out according to protocols as described by U.S. EPA (1998).

### Statistics

The statistical analyses were performed with the SAS statistical software package (SAS V8 and enterprise guide V1) and a significance level of $p$=0.05 was used except where stated otherwise. Contaminant data was log-transformed (base e) prior to the analyses in order to meet the criteria of normality and homogeneity of the variance. The differences in the prevalence of periodontitis among periods, sex and agegroups were tested with $X^2$ tests. The difference in organohalogen loads between the two groups with and without periodontitis was tested by a one-sample F-test.

## Results

### Periodontitis

The age distribution over the entire period from 1892 to 2002 is viewed in Fig. 1 (not the large sampling from 1999 to 2002). In the 272 polar bear skulls that were examined for macroscopic pathological changes, we found cases of periodontitis, mandibulare/frontale osseus proliferations, adonti (single tooth), deplacement of tooth, boneloss posterior to M1 and caries in incisivi, premolar and molar. In old polar bears (≥15 years of age) the severe cases of periodontitis were accompanied by loss of alveolar bone tissue and tooth wear. However, only periodontitis was found in frequencies that allowed for statistical analyses (Fig. 2).

**Figure 1**
Individual age of East Greenland polar bear skulls collected 1892-2002. Subadults (both sexes).

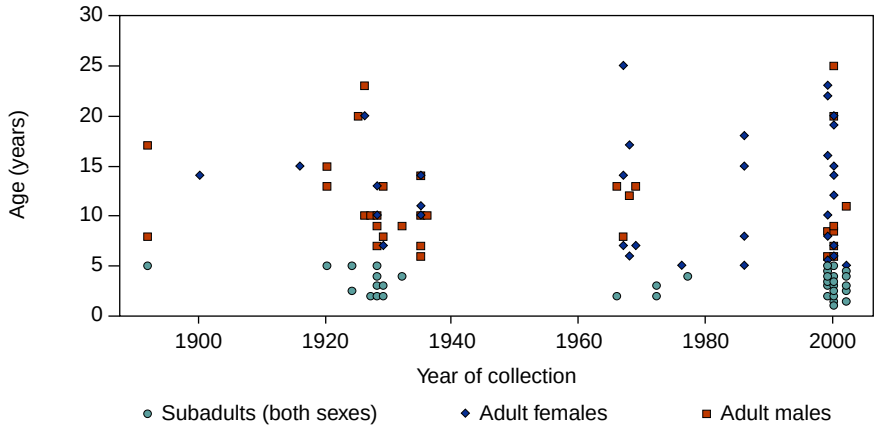
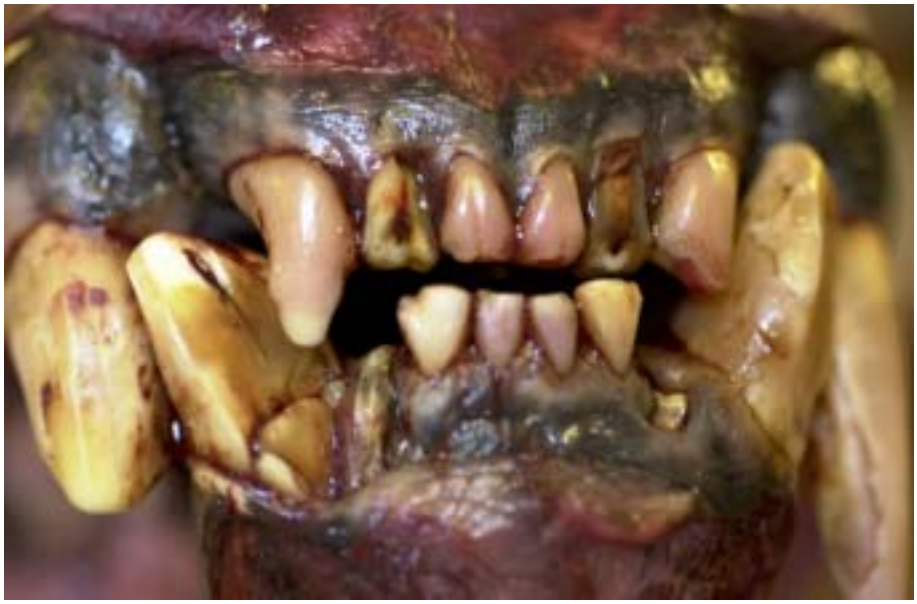
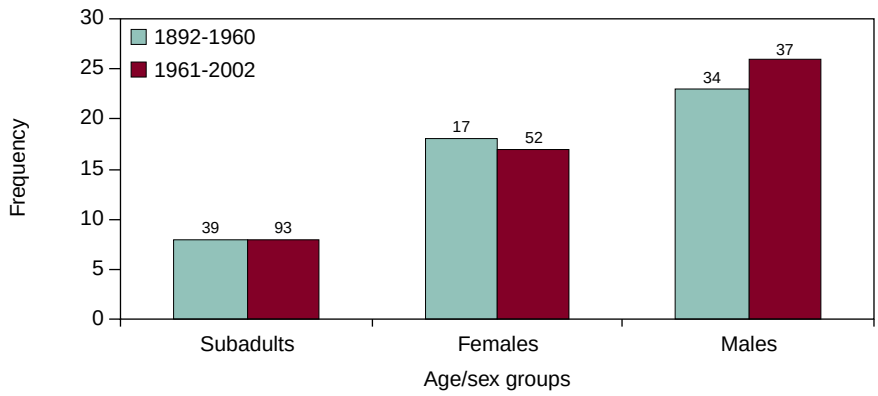



**Figure 2**
Example of periodontitis in a 20-year-old male polar bear (ID #Steen) collected in Scoresby Sound (East Greenland) sampled in the summer 2000. Note the breakage and the open pulp cavity of the lower right canine (arrow) and the loss of alveolar bone tissue. The picture was taken before maceration.



The analysis showed, that the frequency of periodontitis increased significantly with age ($p<0.01$) whereas a sex differences could not be detected ($p>0.2$) (Fig. 3). The prevalence of periodontitis did not differ between the pre-pollution (period 1) and the pollution (period 2) sample sets for any age or sex group (all: $p>0.7$) (Fig. 3).

**Figure 3**
Frequencies of periodontitis in polar bears by age/sex category for two sampling periods (1892-1960 and 1961-2002, respectively). Above bars=n.



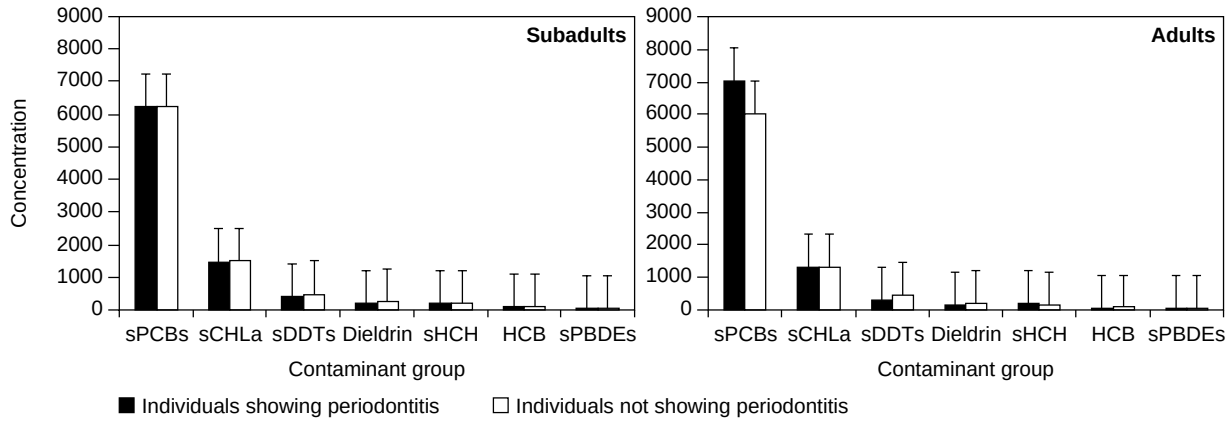



**Figure 4**
Mean (SD) of various organohalogens (ng/g l.w.) in adipose tissue of subadult and adult polar bears sampled in East Greenland, 1999-2002 (n=79). Black bars indicate individuals showing periodontitis (4 subadults and 26 adults, respectively) and white bars indicate bears not showing periodontitis (39 subadults and 10 adults, respectively).

### Organohalogen concentrations

We investigated if there was a difference in the levels of organohalogens between individual showing periodontitis and individuals not having periodontitis within each group of age/sex group (subadults and adults respectively) (Fig. 4). This data explorance showed that there was no statistical significant difference in mean levels of organohalogen compounds in bears with and bears without periodontitis for any of the contaminants analysed (all: $p>0.1$) (Fig. 4).

## Discussion

### Wildlife and periodontitis

In the present study, there was no difference in the frequency of periodontitis between the pollution period and the pre-pollution in any of the age groups (Fig. 3), and the present results are therefore not in agreement with reports of skull bone lesions in harbour seals (*P. vitulina*) and grey seals (*H. grypus*) from Swedish and Danish waters. In these reports, lesions (periodontitis, often with substantial loss of alveolar bone in mandible and maxilla, and alveolar exostosis) were correlated to environmental pollutant levels (Bergman *et al.* 1992, Mortensen *et al.* 1992). Schandorff (1997) also found that for harbour seal (*P. vitulina*) a significant difference in the prevalence of periodontitis between non-polluted and polluted decades. The adipose levels of PCBs and DDTs found in these seal studies were significantly higher than in the present polar bears, which may explain why we did not find a contaminant-related time trend. The peridontitis complex also may not be a sensible parameter for organohalogen exposure. In Table 1 the organohalogen concentrations in seals from Danish and Baltic waters are compared to the present East Greenland polar bears.

**Table 1**
Range in the levels of organohalogenes (µg/g l.w.; blubber) linked to periodontitis in the Kattegat harbour seal (Phoca vitulina) and Baltic grey seal (Halichoerus grypus) (range for juveniles, subadults and adults) from before and around 1988 compared to the range in levels of polar bears from East Greenland in the present study. n: number of observations (data from: Blomkvist et al., 1992; Schandorff 1997a,b and Zakharov and Yablokov 1990).

| Species/variable | Organohalogen compound (n) | Concentration around 1988 | Concentration in adipose tissue in East Greenland polar bears in the present study (n) |
|---|---|---|---|
| Kattegat harbour seal | ∑PCBs (38) | 6-110 (blubber) | 1-20 (77) |
| Kattegat harbour seal | ∑DDTs (38) | 2.0-13 (blubber) | 0.1-1.1 (77) |
| Baltic grey seal | ∑PCBs (37) | 32-5300 (blubber) | 1-20 (77) |
| Baltic grey seal | ∑DDTs (37) | 11.0-1600 (blubber) | 0.1-1.1 (77) |

### Relations between levels of 2,3,7,8-TCDD, CB-126 and periodontitis

In mink (*Mustela vison*) proliferation of periodontal squamous epithelium and osteolysis of alveolar bone structures in mandible and maxilla was induced by CB-126 and 2,3,7,8-TCDD (Render *et al.* 2000a-b, Render *et al.* 2001). In Sonne *et al.* (In press) the TEQ-values of mono-ortho PCB congeners and PCDD/Fs in the present polar bears was investigated. Briefly, CB-126 make up the majority of the total TEQ levels and these were magnitudes lower compared to known thresholds from (marine) mammals. The results reported by Render *et al.* was in agreement with the investigations by Bergman *et al.* (1992), Mortensen *et al.* (1992) and Schandorff (1997a-b) and may be a result of immunosuppression. The CB-126 and 2,3,7,8-TCDD exposure to East Greenland polar bears were significant lower compared to studies by Render *et al.* (Riget *et al.* 2003, Johansen *et al.* 2004). Also CB-126 and 2,3,7,8-TCDD burdens in the fat of East Greenland polar bear was significant lower compared to the levels used in studies by Render *et al.*, which again could explain that we did not find a difference in periodontitis between the non-polluted and polluted periods.

### Periodontitis and tooth wear as mortality factor?

Stirling (1969) investigated tooth wear as a mortality factor in the Weddell seal (*Leptonychotes weddelli*) at the Antarctic. He found that the amount of tooth wear and necrosis increased markedly after app. 9 years of age and that this may contributate markedly to the mortality in adult individuals of this seal species. We cannot evaluate the mortality of the present lesions in the East Greenland polar bear but it is likely that the most severe lesions could contribute to increased mortality due to starvation (pain and inflammation).

## Conclusions

Our results clearly showed that the prevalence of periodontitis increased with age and that no differences was found between adult females and males nor between periods with pollution (1892-1960) and witout pollution (1961-2002). In old animals (≥15 years of age) with severe periodontitis (loss of alveoloar bone tissue), this was accompanied by tooth wear. We did not find a relation between individual levels of organohalogens and the prevalence of periodontitis. We propose that the periodontitis and tooth wear may be a mortality factor in the East Greenland polar bear.

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# Paper IV
# Liver histology of free-ranging East Greenland polar bears (*Ursus maritimus*) in relation to organohalogen exposure

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## Summary

The liver histology of 34 subadult, 29 adult female and 25 adult male polar bears (*Ursus maritimus*) sampled in East Greenland during 1999-2002 were studied to evaluate the toxicity of anthropogenic organohalogens (PCBs, DDTs, CHLs, dieldrin, HCHs, HCB and PBDEs). Lightmicroscopic findings were nuclear displacement from normal, central cytoplasmic location in parenchymal cells, mononuclear cell infiltrations (mainly portally and as granulomas), bile duct proliferations, portal fibrosis and fat accumulation in hepatocytes and prominar Ito-cells. Of these, portal fibrosis was found to be positively correlated with age while bile duct proliferation was highly correlated to the degree of portal fibrosis (some seasonal variations were found as well). Significant differences in mean concentrations of ∑-DDTs was found between groups of histological changes but these were thought to be a result of low sample size. To our knowledge this is a first time evaluation of liver histology in relation to organohalogen concentrations in mammalian wildlife species and the signs of chronic hepatitis, also in relation to Glisson's triads, is probably natural events in the East Greenland polar bear from ageing, infectious agents, lipid hyperphagia and fasting, but long-term exposure to mercury and organohalogen compounds cannot be ruled out as co-factors.

Key words: polar bear, *Ursus maritimus*, East Greenland, PCBs, DDTs, liver, bile duct proliferation, portal fibrosis, lipid granulomas, Ito cells.

## Introduction

In marine wildlife, chronic exposure to organohalogens (*e.g.* PCBs and DDTs) has been associated with toxic effects on several organ systems (Bergman and Olsson 1985, Bergman 1999, Bergman *et al.*, 2001, Schumacher *et*

*al.*,1993). Hepatic changes associated with high environmental levels of organohalogens have only been investigated in cormorants (*Phalacrocorax carbo*) (Fabczak *et al.*, 2000) and fish (*Abramis brama*) (Koponen *et al.*, 2001) and such associations has not been studied in marine mammals.

In laboratory animals like rats and mink, several acute studies of PCBs have associated these compounds with hepato-toxicity due to the portal vein transport and high metabolic capacity of it's parenchymal tissue (*e.g.* Kimbrough *et al.*, 1971, Bruckner *et al.*, 1974, Jonsson *et al.*, 1981, Bergman *et al.*, 1992, Chu *et al.*, 1994, MacLachlan and Cullen 1995, Parkinson 1996). Specifically in the liver, acute organohalogen compound toxicity is mediated through subcellular toxicity (impaired ATP synthesis, protein synthesis a.o.) (Parkinson 1996) while chronic exposure may affect endocrine homeostasis via upregulation of CYP-isozymes (*e.g.* 1A and 1B) (*e.g.* Boon *et al.*, 1992, Wong *et al.*, 1992, Lin *et al.*, 2003, van Duursen *et al.*, 2003).

In Arctic marine wildlife, polar bears (*Ursus maritimus*) from East Greenland are among those polar bears carrying the highest levels of organohalogens and in some individuals, the levels are in the range that are suspected to negatively affect reproduction and survival in seals from the Baltic Sea (de March *et al.*, 1998, AMAP 2004). Liver tissue, subcutaneous fat and skulls were collected from the East Greenland subsistence hunt of polar bears during 1999-2002. The purpose was to study if there was a correspondance between histopathological changes, in the liver and known specific organohalogen induced changes and if there was a correlation between these and individual levels of organohalogens.

## Materials and Methods

Hepatic tissue ($n$=88) and skulls ($n$=44) from East Greenland polar bears from the areas between Skjoldungen at 63º15'N and Danmarks Havn at *ca.* 76º50'N were sampled 1999-2002 by greenland subsistence hunters and deposited at the Zoological Museum in Copenhagen, Denmark. All samples were taken <12 *h post mortem* and preserved in the field during the hunt and later kept at minus 20 ºC before examination in the laboratory in Copenhagen.

Samples of adipose tissue were stored in Poly Ethylene plastic bags until arrival in the laboratory, where they were transferred into rinsed glass containers with lid aluminium foil. Samples of liver were in the field stored in a combination of formaldehyde and alcohol (10% of a 35% formaldehyde solution and 90% of a 96% ethanol solution). Skulls were macerated in the laboratory - and if necessary boiled (<5 min) - so that muscles and tendons could be gently removed prior to $H_2O_2$ oxidation for 24-48 hours.

### Age estimation

The age determination was carried out by counting the cementum Growth Layer Groups (GLG) of the lower $I_3$ after decalcification, thin sectioning (14µm) and staining (toluidine blue) using the method described by *e.g.* Hensel and Sorensen (1980) and Dietz *et al.*, (1991). For statistical analyses, the individuals were categorised as subadults, adult males and adult females, respectively, by the criteria: adult males ≥ 6 years, adult females ≥ 5 years and others as subadults (*e.g.* Roosing-Asvid *et al.*, 2002).

### Histology

All tissue samples were trimmed, processed conventionally, embedded in paraffin, sectioned at about 4 µm and stained with Haematoxylin (Al-Haematein)-Eosin (HE) and periodic acid-Schiff (PAS) for routine diagnostics, Best's carmine to demonstrate glycogen storage and perls' prussian blue reaction and Smorl for detecting haemosiderin and lipofuscin, respectively (Lyon *et al.*, 1991, Bancroft and Stevens, 1996). All slides were examined on a Leica DMLB microscope with 50, 100, 200, 400, 630 and 1000 x magnification and pictures were digitialised by a Leica DC300 digital camera.

Histopathological changes (mononuclear cell infiltrations, granulomas, bile duct proliferation, portal fibrosis, lipid accumulation and necroses) were evaluated semi-quantitatively by examinating the liver tissue in 10 randomly selected low to high power fields (200-400x magnification). Based on these observations each liver tissue was ascribed into one of three groups (absent-mild-moderate). Areas with severe freezing artifacts were omitted from evaluation.

### X-ray (osteodensitometry)

X-ray osteodensitometry (DXA, Norland XR 26) was applied to detect osteoporosis in the skulls of a sub-set of 44 polar bars. The method of determining bone mineral density (BMD) in these bears are described in detail in Sonne *et al.*, (Accepted with revision).

### Analyses of PCBs and OCs

Polar bear adipose tissue samples (*n*=67) were analysed for PCBs (PolyChlorinatedBiphenyls), DDTs (DichloroDiphenylTrichloroethanes), HCHs (HexaCycloHexanes), CHLs (CHLordanes), HCB (HexaChloroBenzene) and dieldrin according to Sandala *et al*. (2004) and Dietz *et al*. (2004) at the Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada. An external standard quantification approach used for PCBs and OCs in the adipose tissues was based on peak area of the GC-µECD response, which is described in detail in Dietz *et al*. (2004). Briefly, ∑-PCBs is the sum (s) of the concentrations of the 51 individual or co-eluting congeners (if detected): CB # 31/28, 52, 49, 44, 42, 64/71, 74, 70, 66/95, 60, 101/84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129/178, 182/187, 183, 128, 174, 177, 171/202/156, 200, 172, 180, 170/190, 201, 203/196, 195, 194, 206. ∑DDTs is the sum of *4,4'*-DDT, *4,4'*-DDD and *4,4'*-DDE. ∑-HCHs is the sum of the α-, β- and γ-hexachlorocyclohexane. ∑-CHLs is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. Contaminant fractions were subsequently sent to the National Water Research Institute (Environment Canada, Burlington, Ontario, Canada L7R 4A6 (NWRI)) for determination of brominated diphenyl ether (PBDE) flame retardants.

### Analyses of PBDEs

Polar bear adipose tissue samples (*n*=68) were analysed for BDPEs by electron capture negative ion (low resolution) MS using an external standard. Briefly, ∑-PBDEs is the sum (s) of the concentrations of the 35 individual or co-eluting congeners (if detected): BDE# 10, 7, 11, 8, 12/13, 15, 30, 32, 28/33, 35, 37, 75, 71, 66, 47, 49, 77, 100, 119, 99, 116, 85, 155/126, 105, 154, 153, 140,

138, 166, 183, 181, 190. Gas chromatographic conditions for the PBDEs were described by Luross *et al.*, (2002).

### Statistics

The statistical analyses were performed with the SAS statistical software package (SAS V8 and enterprise guide V1) and a significance level of $p$=0.05 was used except where stated otherwise. The BMD showed no deviation from normality (Shapiro-Wilk test) while contaminant data were log-transformed (base e) prior to the analyses in order to meet the assumption of normality and homogeneity of the variance.

#### Histology in relation to age, sex and season

The relationship between grades of histopathological changes (absent, mild and moderate, respectively) and age was tested within a one-way ANOVA (age as dependent variable and histopathological change as independent). Relations between histological changes (mononuclear cell infiltrations, granulomas, bile duct proliferation and portal fibrosis), sex and season were tested within $X^{2}$-tests.

#### Relationship between histology and contaminants

The relationships between each group of contaminants (PCBs, DDTs, HCHs, CHLs, HCB, PBDEs and dieldrin respectively), histology (lipid accumulation, portal fibrosis, bile duct proliferations and cell infiltrations) and age / sex (subadults, adult females and adult males) were tested in a two-way ANOVA (contaminant group as dependent variable and age / sex group and histology as class varia-bles) with 1st order interaction links (age / sex group*histological changes). The principle in the further data handling was a successive reduction of non-significant interactions, judged from the type-III sum of squares ($p$>0.05), and the significance of the remaining factors was evaluated from the final model (LSMean). When significant differences between age / sex groups were found, these were tested among each other by Tukey's *post hoc* test.

#### Relationship between histology and skull BMD

The principle in the data handling for subadults, adult females and adult males respectively, was a model with BMD (bone mineral density) as dependent variable, age as covariable variable, histology as class variable and their 1st order interaction links (age*histology). The model was successively reduced for non-significant interactions ($p$>0.05) justed from the type-III sum of squares and the significance of the remaining factors were evaluated from the final model (LSMean).

## Results

Hepatic tissue from 34 subadults, 29 adult females and 25 adult males was examined histologically (of these bears, skulls were available for BMD measurement in 44 individuals, adipose organochlorine concentrations in 67 individuals and adipose PBDEs concentrations in 68 individuals, respectively). Generally, the polar bear liver consisted of lobuli based on sinusoids, while interlobular fibrous septa seemed to lack as in other Ursid species (MacLachlan and Cullen 1995, Frappier 1998, Prunescu *et al.*, 2003). Kupffer-

cells, located in the space of Disse, were tested positive for haemosiderin (iron pigments) and hepatocytes were tested positive for deposits compatible with glycogen. In addition, nearly all individuals exhibited nuclear dislocation from the normal, central cytoplasmic placement in parenchymal cells (Fig. 1). In the following, each histological change is described separately.

## Lipid

The majority (81%) of animals showed lipid accumulation in hepatocytes and prominent Ito-cells of varying degree (Table 1, Fig. 1). Hepatocytic accumulation was present as microvesicular lipid seen as foamy cytoplasm or as sharply demarcated vacuoles, and distributed mainly centrolobularily. Non-parenchymal lipid vacuoles of diverging size and number were found in centroacinary pluripotent Ito-cells (*i.e.* stellate cells and lipocytes), located in the narrow space of Disse and between hepatocytes, mainly in centroacinary zones (Fig. 1). In the case of intra-hepatocytic lipid accumulation (micro- and macrovesicularily, respectively) there was no difference among age/sex group ($p>0.3$), while the degree of Ito-cell lipid accumulation was highly different among age/sex groups ($p<0.001$) increasing in the order adult males<adult females<subadults (Table 1, Fig. 1).

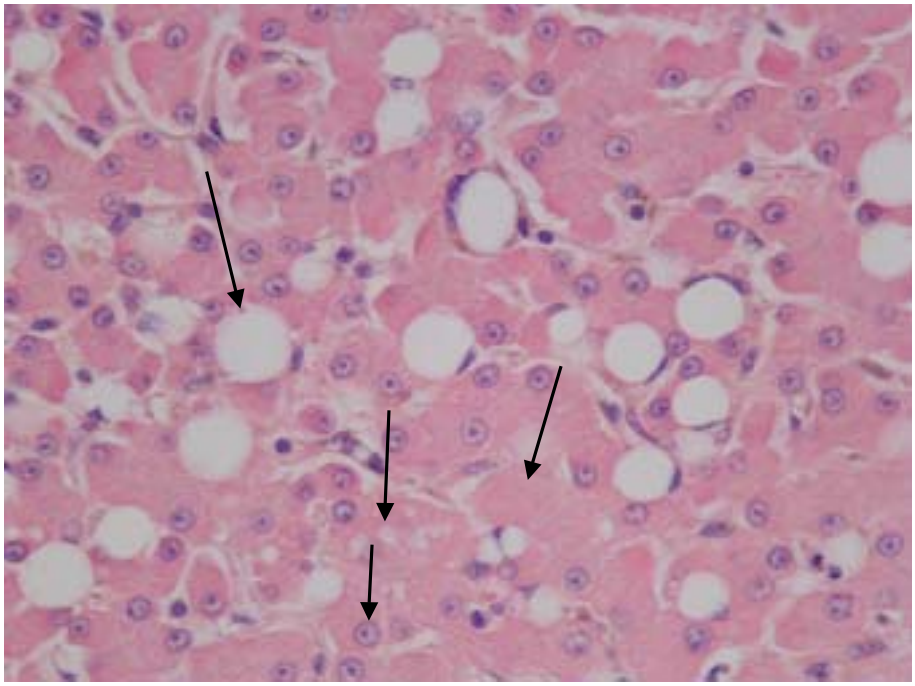

**Figure 1**
Hepatic lipid accumulation in a 16-year-old female East Greenland polar bear (group: moderate). Arrows indicate cites of microvesicular intracellular fat (foamy cytoplasm), macrovesicular intrahepatocytic cellular fat, Ito-cell lipid accumulation and periorientation of the nuclei (HE, 40x).

**Table 1**
Results of histological examination of liver tissue from 88 polar bears sampled in East Greenland, 1999-2002 given as frequencies (no. of observations in paranthesis). Changes considered were lipid content of Ito-cells (lipid), portal mononuclear cell infiltrations (infiltrations), lipid granulomes (granulomes), portal fibrosis (fibrosis) and bile duct proliferation (proliferation). Histopathological findings were classified according to severity (absent, mild and moderate). Sub: subadults of both sexes, AdF: adult females and AdM: adult males. In few cases of autolysis and freeze damage, one or more of the changes considered could not be evaluated.

| Group | Lipid | | | Infiltrations | | | Granulomes | | | Fibrosis | | | Proliferation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sub | AdF | AdM | Sub | AdF | AdM | Sub | AdF | AdM | Sub | AdF | AdM | Sub | AdF | AdM |
| Absent | 41% (14) | 10% (3) | 0% (0) | 3% (1) | 8% (2) | 21% (5) | 16% (5) | 52% (13) | 33% (8) | 55% (17) | 48% (12) | 33% (8) | 68% (21) | 56% (14) | 58% (14) |
| Mild | 26% (9) | 10% (3) | 16% (4) | 66% (21) | 72% (18) | 54% (13) | 68% (21) | 36% (9) | 54% (13) | 45% (14) | 52% (13) | 67% (16) | 32% (10) | 44% (11) | 42% (10) |
| Moderate | 32% (11) | 80% (23) | 84% (21) | 31% (10) | 20% (5) | 25% (6) | 16% (5) | 12% (3) | 13% (3) | . | . | . | . | . | . |
| Total | 34 | 29 | 25 | 32 | 25 | 24 | 31 | 25 | 24 | 31 | 25 | 24 | 31 | 25 | 24 |

### Mononuclear cell infiltrations and lipid granulomas

The second histological change was focal, mainly portal, mononuclear cell infiltrations (lymphocytes, macrophages and neutrophils) and in few cases necrosis (eosinophilic bodies) of individual hepatocytes (chronic multifocal non-suppurativ hepatitis) (Fig. 2) and 1 case of fibrin exudation (focal acute hepatitis). In cases with heavy cell infiltrations these were randomly distributed (disseminated). The degree (absent, mild and moderate) of portal mononuclear cell infiltrations was not age-related ($p>0.4$), and did not differ between subadults and adults ($p>0.4$, Table 1). Additionally, granulomas were noted, and frequently containing a central vacuole (lipid granulom) although the vacuole could represent necrotic heaptocyte as well as lipid. Individual exhibiting moderate granuloma score were signficant older compared to individuals having mild granuloma score ($p=0.005$). There was a tendency – although not statistically significant - of subadults having a higher frequency of granulomas than adults ($p<0.08$, Table 1). Finally, livers with Ito-cell intracellular demarcated fatty vacuoles showed a larger frequency of fatty granulomas with central rarefaction compared to livers without fatty Ito-cells. However this difference was not statistically different ($p<0.06$).

The histology of renal tissue was also examined in the present polar bears, but reported elsewhere (Sonne *et al.*, submitted). In cases of mononuclear cell infiltrations in the liver, these were also found in the cortex, medulla and / or papilla of the kidneys of the same individual in most cases, and this relationship was significant ($p<0.05$).

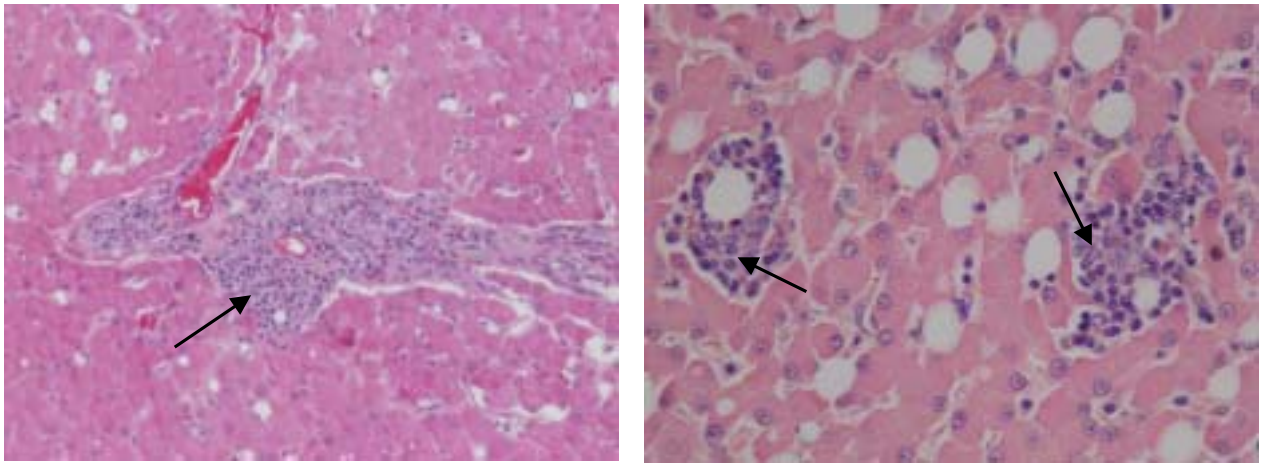

**Figure 2**
Left: Portal mononuclear cell infiltrations in a 3.5 year-old female polar bear (HE, 10x). Right: centroacinary lipid granulomas in a 16 year-old female East Greenland polar bear (same individual as in Fig. 1) (HE, 40x).

### Portal fibrosis and bile duct proliferation

Portal fibrosis was correlated to age ($p$<0.02). The portal cell infiltrations were frequently accompagnied by mild portal fibrosis (Fig. 3) and this relationship was found to be significant ($p$<0.04). In addition, mild bile duct proliferation was seen (Fig. 3) and found highly related to portal fibrosis ($p$<0.001). No difference was found among age/sex groups for bile duct hyperplasia (p>0.5) while indications of a higher frequency positive age related portal fibrosis was found, however not statistically significant ($p$=0.08) (Table 1).

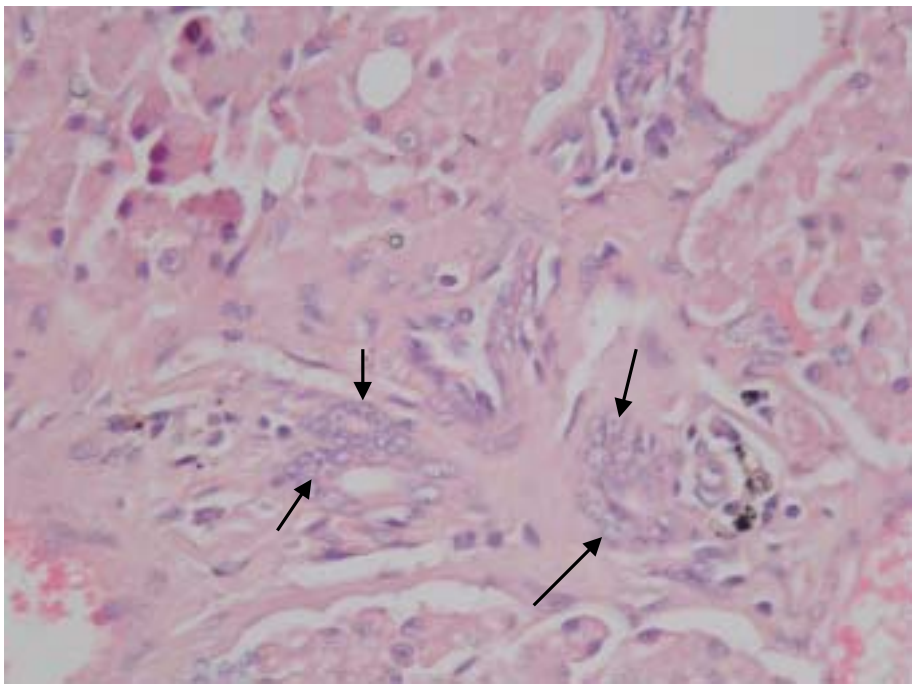

**Figure 3**
Portal area of a 16-year-old female East Greenland polar bear (same individual as in Fig. 1). Arrows indicate mild bile duct proliferation (HE, 40x).

### Seasonal variation

It was investigated if histological changes were related to season. Seasonal changes in *e.g.* food resources (*i.e.* winter versus summer) could perhaps influence liver histology. However, the degree of Ito-cell development (lipid accumulation) did not differ between periods of high food resource a-

vailability (Apr-Jul) and low (Aug-Oct) ($p$>0.11). But there was a larger hepatocytic lipid content for subadults (both micro- and macro-vesiculary) in the bears sampled in Apr-Jul when compared to bears from Aug-Oct ($p$=0.05). Adult bears from Jan-Jun (but not for Apr-Jul) showed higher frequency of portal fibrosis, when compared to adult bears from Aug-Oct ($p$<0.02). No seasonal difference was found for bile duct proliferations ($p$>0.28), and neither for mononuclear cell infiltrations nor lipid granulomas (both: $p$>0.25).

### Relationship between histology and contaminants

In the comparison of contaminant levels and histopathological changes, individuals were categorised into three groups (absent, mild and moderate) based on the severity of histological changes (Table 2). ∑-DDTs mean levels differed between pathology groups for adult females ($p$<0.05). However, the contaminant levels increased in the order mild<moderate<absent so no clear pattern was found. The ∑-PBDEs differed between pathology groups for adult males only ($p$<0.001), with ∑-PBDEs decreasing with the severity of the histological changes, which could reflect a decrease in the ability of the liver to store OHCs. Organochlorine levels in relation to age, sex and season is reported in Dietz *et al.,* (2004).

**Table 2**
Mean (SD) concentrations of organohalogens (ng/g l.w.) in subcutaneous adipose tissue of 68 subadult, adult female and adult male East Greenland polar bears investigated for liver lesions during 1999-2002. The concentrations are divided on liver histological changes (absent, mild and moderate, respectively) based on the results from Table 1. No. of samples are given in paranthesis. "." indicates missing data. *: indicates statistical significant differences in organohalogen levels between the three means of the specific age/sex group at 5% level.

| Contaminant group | Histopathology | Subadults | Adult females | Adult males |
|---|---|---|---|---|
| ∑-PCBs | Absent | . | 2704±267 (2) | 9083 (1) |
| | Mild | 6119±3367 (26) | 6060±4023 (17) | 7252±3315 (15) |
| | Moderate | 5974 (1) | 6228±3442 (3) | 5392±976 (2) |
| ∑-DDTs | Absent | . | 705±361 (2)* | 328 (1) |
| | Mild | 439±202 (26) | 339±170 (17)* | 525±259 (15) |
| | Moderate | 1151 (1) | 355±21 (3)* | 291±231 (2) |
| ∑-CHLs | Absent | . | 719±176 (2) | 1522 (1) |
| | Mild | 1517±1018 (26) | 1701±1537 (17) | 1057±597 (15) |
| | Moderate | 1855 (1) | 1290±532 (3) | 703±299 (2) |
| Dieldrin | Absent | . | 179±23 (2) | 280 (1) |
| | Mild | 215±117 (26) | 185±68 (17) | 184±93 (15) |
| | Moderate | 182±70 (1) | 163±23 (3) | 90±43 (2) |
| ∑-HCHs | Absent | . | 127±3 (2) | 336 (1) |
| | Mild | 185±74 (26) | 194±169 (17) | 224±147 (15) |
| | Moderate | 144 (1) | 166±42 (3) | 132±59 (2) |
| HCB | Absent | . | 28±4 (2) | 97 (1) |
| | Mild | 112±105 (26) | 80±73 (17) | 49±31 (15) |
| | Moderate | 136 (1) | 48±27 (3) | 30±10 (2) |
| ∑-PBDEs | Absent | . | 42±6 (2) | 156 (1) |
| | Mild | 58±33 (26) | 61±38 (18) | 48±16 (15)* |
| | Moderate | 50 (1) | 59±23 (3) | 21±6 (2)* |

### Relationship between histology and BMD

We measured the bone mineral density (BMD) within groups of histology (absent, mild and moderate) in 44 subadult, adult female and adult male polar bears (Table 3). The sample size for subadults and adult males were too small to evaluate the relationship between histopathological changes and BMD. For adult females, there was no significant difference in BMD levels between groups of mild and moderate histopathological changes. Levels, age relations and the negative correlations between ∑-PCBs, ∑-DDTs, dieldrin and ∑-CHLs, respectively, and skull BMD are reported in Sonne *et al.,* (Accepted with revision).

**Table 3**
Mean (SD) levels of of Bone Mineral Density (BMD; g hydroxyapatite/cm$^2$) in the skull of 44 East Greenland polar bears investigated for liver lesions during 1999-2002. The concentrations are divided on age/sex groups (subadults, adult females and adult males respectively) and liver histological changes based on the results from Table 1. Number of samples are given in paranthesis. "." indicates missing data. $^{n.s.}$ indicate no statistical significant differences between the three means of the specific age/se $^{s}$. x group at 5% level. $^{‡}$ could not be evaluated due to low sampling size.

| Histopathology | Subadults$^{‡}$ | Adult females$^{n.s.}$ | Adult males$^{‡}$ |
|---|---|---|---|
| Absent | . | . | 2.2 (1) |
| Mild | 1.7±0.4 (19) | 1.9±0.1 (10)$^{n.s.}$ | 2.3±0.3 (10) |
| Moderate | . | 2±0.2 (3)$^{n.s.}$ | 2.6 (1) |

## Discussion

Nuclear dislocation was found in nearly all individuals. Studies by Sato *et al.,* (2001) of polar bears from Svalbard revealed the same, and it was proposed that it could be related to the high vitamin A accumulation (natural storage) in Ito-cells, accumulated through the "fatty" Arctic marine food chains. In mammalian species, the dislocation is usually associated with hepatitis, carcinomas, hyperplasia (adenomatous) or regeneration (Ibid.). However, in the study by Sato *et al.,* (2001) such histopathological changes were not found. Whether the histological changes in the East Greenland polar bears could be associated to the nuclear dispositioning, could not be evaluated from our study because all individuals exhibited some or all of the histopathological changes. If there was a link between nuclear dislocation and organohalogens or hepatocytic lipid accumulation (*e.g.* MacLachlan and Cullen 1995) could not be evaluated either.

### Lipid

The Ito-cells are the accumulation and storage site for lipids and vitamin A in polar bears (Leighton *et al.,* 1988, Senoo *et al.,*1999, 2001). In the present study, fat accumulation (lipids) were found in hepatocytes and Ito-cells (centrolobularily and centroacinary respectively). Polar bears are hyperphagiae from April to July, where they build up their fat deposits (Ramsay and Stirling 1988; Messier *et al.,* 1992). Based on samples from the same polar bears as used in the present study, Dietz *et al.,* (2004) showed a highly seasonal pattern in the lipid-% in adipose tissue, with the lowest levels in spring (Mar-May) during hyperphagia and the highest during Sept. In the present investigation, the seasonal difference in hepatocytic lipid content of subadults (Apr-Jul higher than Aug-Oct) probably reflected the high seal blubber consumption in spring (Ramsay and Stirling 1988; Messier *et al.,* 1992).

### Mononuclear cell infiltrations and lipid granulomas

Mononuclear cell infiltrates - accompanied by fibrosis – might be reactions due to local depositioning of bacteria, virus or injury of local blood vessels (MacLachlan and Cullen 1995). The strong indications of predispositioning of lipid granulomas around swollen Ito-cells, located in the narrow space of Disse and between hepatocytes, might be explained by infectious agens originating from the blood supply, leading to random focal necroses (burst of Ito-cells) (*Ibid.*).

### Portal fibrosis and bile duct proliferation

Age related portal fibrosis due to chronic infections (cholangitis and biliary obstruction) is a common feature in mammals (MacLachlan and Cullen 1995) and it has been reported in the romanian brown bear (*U. arctos*) (Prunescu *et al.*, 2003) and Arctic beluga whales (*Delphinapterus leucas*) (Woshner *et al.*, 2002). In Prunescu *et al.*,'s study, seasonal liver fibrosis (highest in spring) of the hepatic venous system was shown, and they speculated whether this was due to pre-hibernation physiological adaptations (*Ibid.*). This finding was in agreement with our study, although only pregnant polar bears den for a long time, whereas all other polar bears of both sexes and age only now and then use shelters (faculative dens), when environmental conditions are adverse (*e.g.* Ferguson *et al.*, 2000). Bile duct proliferation due to hepatic injury has been related to liver injury and is as such a non-specific histopathological change also described in Arctic beluga whales (*Delphinapterus leucas*) (MacLachlan and Cullen 1995, Wosher *et al.*, 2002). The aetiology of chronic lymphocytic cholangitis is unknown but toxic injury, parasitism or periductular fibrosis has been proposed (MacLachlan and Cullen 1995).

### Histology and contaminants

To our knowledge, liver histology in relation to environmental levels of organohalogens has only been studied in cormorants (*Phalacrocorax carbo*) (Fabczak *et al.*, 2000) and fish (*Abramis brama*) (Koponen *et al.*, 2001) but not in mammals. Therefore it is hard to evaluate the relationship between liver histology and chronic exposure to environmental levels of organohalogen compounds in the East Greenland polar bear. Liver toxic substances (*e.g.* copper, pyrrolizidine alkaloids, carbon tetrachloride and phytotoxins) usually produce a centrolobulary (periacinary) zone 3 characteristic injury due to the low oxygen gradient (hypoxia) and high concentrations of *e.g.* Cyt-P450 isozymes (activation of reactive metabolites) of this zone (MacLachlan and Cullen 1995, Parkinson 1996). In the case of hepatocytic and Ito-cell fat accumulation in our study, these were often concentrated centrolobularily or centroacinary, respectively. Abnormal amounts of fat is known to be accumulated in the liver during high lipid ingestion, starvation, abnormal hepatocytic function, excessive dietary intake of carbohydrates and decreased syntheses of apoproteins and thereby lipoproteins (*Ibid.*). Hence the large content of lipids in polar bear livers could be a function of hyperphagia and starvation due to seasonal changes in food resources as discussed in previous sections. However, acute toxic investigations in laboratory rats of PCBs, DDTs and dieldrin have shown to induce high lipid accumulation - probably due to decreased production of lipoproteins through impaired ATP synthesis and protein synthesis – centrolobularily in hepatocytes (foamy cytoplasm or large vacuoles) (*e.g.* Kimbrough *et al.*, 1971, Kimbrough *et al.*, 1972, Bruckner *et al.*, 1974, Bergman *et al.*, 1992, MacLachlan and Cullen 1995, Par-

kinson 1996). Due to these mechanisms we cannot reach any firm conclusions on whether or not organohalogens have any impact on liver lipid accumulation in polar bears.

Mononuclear cell infiltrates (lymphocytes and neutrophils) randomly distributed (lipid granulomas) or portally (around triads) were associated to subacute toxicity of PCBs in mink (*Mustela vison*) studies (Bergman *et al.*, 1992). We found the same pattern in polar bears, which supports the hypothesis that organohalogens could have been responsible for some of the histopathological changes we found. Although the results from the laboratory studies are non-specific reactions, there are parallels to our findings in East Greenland polar bears. The signs of chronic inflammation, also in relation to Glisson's triads (fibrosis and bile duct proliferation), as well as the lipid accumulation could possibly indicate long-term exposure to tissue toxic substances but as stated above the findings could be natural events in the East Greenland polar bear as well. The differences in DDT and PBDE levels between groups of histopathology for adult females and males, respectively, was not consistent and was probably a result of the relatively low sample size and high number of tests. Meanwhile, it could be speculated if the adipose organohalogen concentration reflect the liver organohalogen burden at all (Dietz *et al.*, 2004).

Beside these considerations, studies of free-ranging Atlantic bottlenose dolphin (*Tursiops truncatus*) (Rawson *et al.* 1993) and Arctic beluga whale (*Delphinapterus leucas*) (Woshner *et al.* 2002) liver histology in relation to mercury exposure have shown similar changes as in the present study and therefore this co-factor cannot be ruled out.

## Conclusions

The histology of polar bear liver tissue was similar to mammal liver tissue in general. In nearly all individuals, nuclear deviations from the normal, central cytoplasmic location in parenchymal cells was found. Mild and moderate changes of lipid content, portal mononuclear cell infiltrates (rarely randomly, granulomas (often lipid granulomas), portal fibrosis and bile duct proliferation, was found. Of these, only portal fibrosis was age-related. The lipid content in Ito-cells was higher in subadults compared to adults, and subadults had a higher frequency of lipid granulomas when compared to adults. Additionally, portal cell infiltrations were frequently accompanied by portal fibrosis and this was highly related to bile duct proliferation. Seasonal variation was found in hepatocytic lipid content (subadults) and fibrosis (adults) both being lower in Aug-Oct compared to the rest of the year. Although no clear relationship between histology and individual levels of organohalogens was found, there were parallels between acute toxicity studies on laboratory mammals and our findings in East Greenland polar bears. The signs of chronic inflammation, also in relation to Glisson's triads (fibrosis and bile duct proliferation), is probably natural events in the East Greenland polar bear from ageing, infectious agents, lipid hyperphagia and fasting, but long-term exposure to mercury and organohalogen compounds cannot be ruled out as co-factors.

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# Paper V
# Renal lesions in East Greenland polar bears (*Ursus maritimus*) sampled during 1999-2002.

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## Abstract

Polar bears from East Greenland are among the most organohalogen polluted bears in the Arctic, and in some individuals the levels are in the range believed to induce adverse health effects. To determine whether this exposure had contributed to adverse effects on internal organs, renal and adrenal tissue were examined light microscopically from 91 and 43 bears, respectively. Specific histologically changes were membraneous glomerulonephritis and -sclerosis - often accompanied with tubular atrophy and hyalinisation – while tubular cellproliferation was recorded in one individual. These histopathological changes were positive correlated to age and were similar to those found in highly PCB and DDT polluted seal populations in the Baltic, and in PCB exposed laboratory animals and occupational exposed Man. No histopathology was found in the adrenals, while relationships between bone mineral density, tubular droplets and pigments and renal lesions, respectively, indicated glomerular and tubular dysfunctions. We therefore suggest that the renal lesions found in the polar bears were a result of ageing and inflectious agebts but long-term exposure to nephrotoxic heavy metals (Cd, Hg) and organohalogen compounds cannot be ruled out as co-factors.

Key words: Polar bear, *Ursus maritimus*, East Greenland, DDTs, PCBs, kidney, glomerulonephritis, tubular lesions, interstitial nephritis, adrenal.

## Introduction

Polar bears (*Ursus maritimus*) from East Greenland, Svalbard and the Kara Sea carry higher loads of anthropogenic organohalogen compounds (*e.g.* PCBs; polychlorinated biphenyls) than polar bears elsewhere in the Arctic (*e.g.* Bernhoft et al., 1997; Norstrom et al., 1988, 1998; de March et al., 1998; Andersen et al., 2001; AMAP, 2004) as these three areas receive air- and sea-born pollutants from the same lower latitudinal sources. PCB levels this high are in the range believed to negatively affect reproduction and survival of seals in the Baltic Sea (*e.g.* de March et al., 1998; AMAP, 2004; Bergman and Olsson, 1985; Swart et al., 1994; Ross et al., 1995, 1996, ). Organohalogens are transferred transplacentally from mother to fetus in mammals (*e.g.* Bernhoft et al., 1997; Polischuk et al., 2002) and act as agonists and/or antagonists to hormones in endocrine organs and tissues (de March et al, 1998; AMAP, 2004; Colborn et al., 1993; Damstra et al., 2002).

In organohalogen dose-response and case-control experiments, toxic effects on renal and adrenal histology have been found in rats (Bruckner et al., 1974a, b; McCormack et al., 1978; Brandt et al., 1992; Wade et al., 2002), bream fish (*Abramis brama*) and asp fish (*Aspius aspius*) (Koponen et al, 2001). Studies of free-ranging grey seals (*Halichoerus grypus*) and ringed seals (*Phoca hispida*) from the Baltic (Bergman and Olsson, 1985; Bergman et al, 2001) have shown an association between organochlorines and renal and adrenal lesions. Damage to the proximal kidney tubules and adrenals can induce demineralisation of the skeletal system (Fanconi's syndrome) leading to renal osteodystrophy (osteoporosis) (Friberg et al., 1986; WHO, 1992). Specific to polar bears, recent studies on PCB levels in bears from Svalbard indicated endocrine disruption of plasma testosterone in males, plasma progesterone in females, plasma retinol (vitamin A) and T4 (Skaare et al., 2001; Haave et al., 2003; Oskam et al., 2003). Additionally, high levels of organochlorines were associated with low levels of IgG, suggesting possible immunotoxic effects (Bernhoft et al., 2000; Lie et al., 2004, submitted).

Therefore samples of renal tissue from 91 bears and adrenals from 43 were collected from East Greenland polar bears (*Ursus maritimus*) through local subsistence hunters in the municipality of Scoresby Sound, 1999-2002. These samples were examined histologically and correlated to individual levels of organohalogens and to skull bone mineral density in a sub-set of 60 bears, to evaluate the potentially chronic toxicity of these.

## Materials and Methods

### Sampling and preparation

Renal ($n$=91), adrenal ($n$=43) and subcutaneous adipose tissue ($n$=39) as well as skulls ($n$=60) were sampled from polar bears by local subsistence hunters in the Ittoqqortoormiit/Scoresby Sound area in central East Greenland (69˚00'N to 74˚00'N) in 1999-2002. All samples were taken <12 *h post mortem* and preserved during the hunt and later kept at minus 20 ºC before preparation and examination in the laboratory in Copenhagen.

Samples of kidney and adrenals were stored in a combination of formaldehyde and alcohol (3.5% formaldehyde, 86% ETOH). Samples of subcutaneous adipose tissue were stored frozen in separate PE plastic bags until arrival in the laboratory, where they were transferred into cleaned glass con-

tainers covered with aluminium foil inbetween the sample and plastic lid. Skulls were macerated in the laboratory - and if necessary lightly boiled (<5 min) - so muscles and tendons could be removed prior to $H_2O_2$ oxidation for 24 to 48 hours.

### Age estimation

The age determination was carried out by counting the cementum Growth Layer Groups (GLG) of the lower $I_3$ tooth after decalcification, thin sectioning (14µm) and staining (toluidine blue) using the method described by *e.g.* Hensel and Sorensen (1980) and Dietz et al. (1991). When necessary the individuals were categorised in subadults, adult males and adult females by the criteria: adult males ≥ 6 years, adult females ≥ 5 years and the remaining as subadults (Rosing-Asvid et al., 2002). In the evaluation of sex differencies in the prevalence of renal lesions adult males and females were categorised as old when they were ≥ 15 years based on Derocher and Stirling (1994).

### Histology

All tissue samples were trimmed, processed conventionally, embedded in paraffin, sectioned at about 4 µm and stained and examined with Haematoxylin (Al-Haematein)-Eosin (HE) for routineously diagnostics, Periodic Acid-Schiff (PAS) and Periodic Acid Silver Methenamine (PAS-M) to demonstrate glomerular basemembrane and mesangial changes, Van Gieson and Masson Trichrom to detect fibrous tissue (collagen) and Smorl and perls' prussian blue reaction to detect lipofuscin and haemosiderin pigments, respectively (Lyon et al. 1991). All slides were examinated on a Leica DMLB microscope with 50, 100, 200, 400, 630 and 1000 x magnification and pictures were digitilised by using a Nikon Coolpix 4500 and a Leica DC300 digital camera.

Glomerular, tubular and interstitial histopathological changes were evaluated semi-quantitatively by examinating the renal tissue in 10 randomly selected low to high power fields (200-400x magnification) and finally ascribe each change into one of three groups (mild-moderate-severe). Areas with severe freezing artifacts were omitted from evaluation.

### X-ray (osteodensitometry)

X-ray osteodensitometry (DXA, Norland XR 26) was applied to detect osteoporosis in the skulls of a sub-set of 60 polar bears. The method of determining bone mineral density (BMD) in these bears are described in detail in (Sonne et al., accepted with revision).

### Contaminant analyses

#### PCBs and OCs

Polar bear subcutaneous adipose tissue samples (*n*=79) were analysed for PCBs (PolyChlorinatedBiphenyls), DDTs (DichloroDiphenylTrichloroethanes), CHLs (CHLordanes), dieldrin, HCHs (HexaCycloHexanes) and HCB (HexaChloroBenzene) according to Sandala et al. (2004) and Dietz et al. (2004) at the Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada. An external standard quantification approach used for PCBs and OCs in the subcutaneous adipose tissues was based on peak area of the GC-µECD response, which is described in detail in Luross et al., (2002).

Briefly, ∑PCBs is the sum (s) of the concentrations of the 51 individual or co-eluting congeners (if detected): CB # 31/28, 52, 49, 44, 42, 64/71, 74, 70, 66/95, 60, 101/84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129/178, 182/187, 183, 128, 174, 177, 171/202/156, 200, 172, 180, 170/190, 201, 203/ 196, 195, 194, 206. ∑DDTs is the sum of *4,4'*-DDT, *4,4'*-DDD and *4,4'*-DDE. ∑HCHs is the sum of the α-, β- and γ-hexachlorocyclohexane. ∑CHLs is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. Contaminant fractions were subsequently sent to the National Water Research Institute (Environment Canada, Burlington, Ontario, Canada L7R 4A6 (NWRI)) for determination of brominated diphenyl ether (PBDE) flame retardants.

#### PBDEs

BDPEs ($n$=80) were determined by electron capture negative ion (low resolution) MS using an external standard. Briefly, ∑PBDEs is the sum (s) of the concentrations of the 35 individual or co-eluting congeners (if detected): BDE# 10, 7, 11, 8, 12/13, 15, 30, 32, 28/33, 35, 37, 75, 71, 66, 47, 49, 77, 100, 119, 99, 116, 85, 155/126, 105, 154, 153, 140, 138, 166, 183, 181, 190. Gas chromatographic conditions for the PBDEs were described by Luross et al., (2002).

#### Statistics

The statistical analyses were performed with the SAS statistical software package (SAS V8 and enterprise guide V1) and a significance level of $p$=0.05 was used. The contaminant data were log-transformed (base e) prior to the analyses in order to meet the assumption of normality and homogeneity of the variance, while BMD showed no deviation from normality (Shapiro-Wilk test).

#### Histology in relation to age, sex, protein casts and season

The relationship between grades of histopathological changes (mild, moderate and severe, respectively) and age was tested within a one-way ANOVA (age as dependent variable and histopathological change as independent). When significant differences were found these were tested among each other by Tukey's *post hoc* test. Then, the prevalence of grades of histopathology between males and females was tested within a $X^2$-test on categorised age groups (subadults, adults and old, respectively). The relationship between grades of histology, season (Oct-Mar vs. Apr-Sept) and medullary hyaline casts, respectively, was tested within a $X^2$-test.

#### Histology and contaminants

The relationship between each group of contaminants (PCBs, DDTs, CHLs, dieldrin, HCHs, HCB and PBDEs, respectively) and histology (mild, moderate and severe) was tested within a two-way ANOVA (contaminant group as dependent variable and age/sex group and histology as class variables) with 1$^{st}$ order interaction links (age/sex group*histological changes). The principle in the further datahandling was a successively reduction of non-significant interactions, judged from the type-III sum of squares ($p$>0.05), after which the significance of each of the remaining factors was evaluated from the final model (LSMean). When significant differences was found between age/sex groups (subadults, adult females and adult males, respectively) in the model, these were tested among each other by Tukey's *post hoc* test.

#### Skull BMD and histology

The relationship between BMD and histology was tested for subadults, adult females and adult males, respectively. The model was a ANCOVA with BMD (bone mineral density) as dependent variable, age as covariable variable, histology as class variable and their 1st order interaction links (age* histology). The model reduction and identification of significant factors were similar to those described in the previous section.

## Results

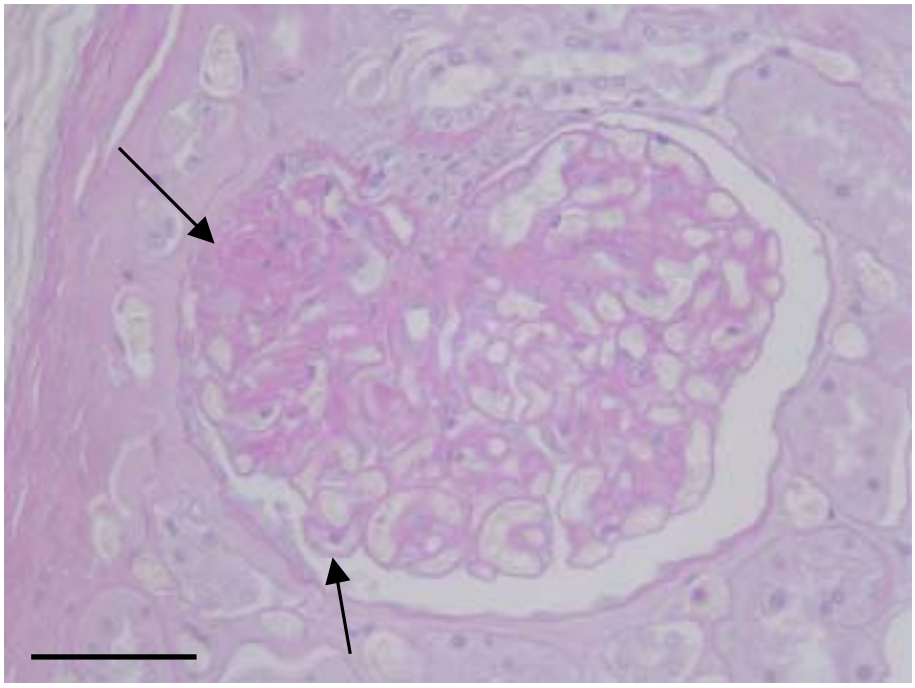
### Kidney

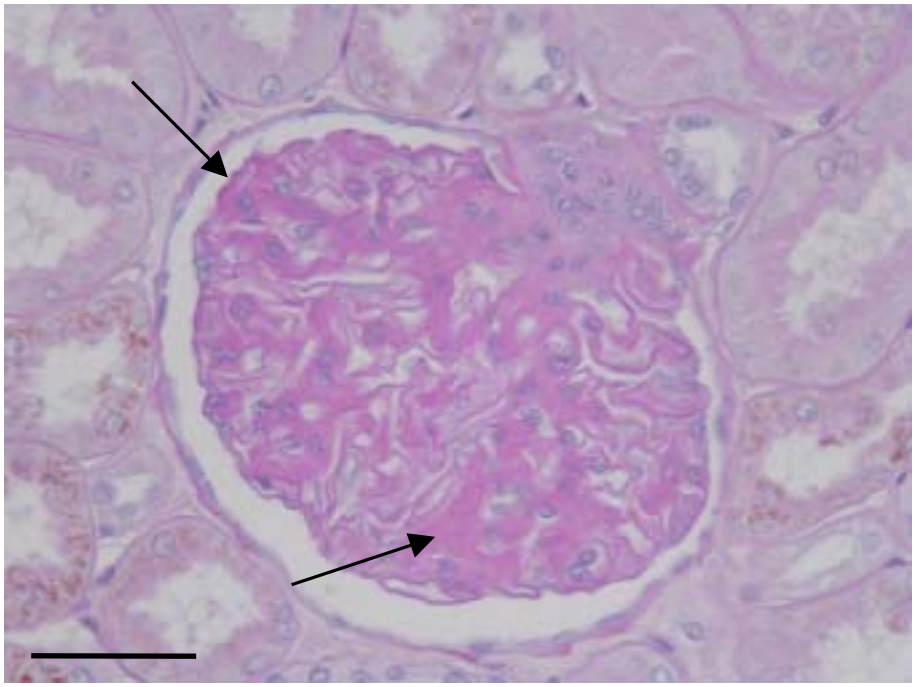
The kidney histology of the 91 polar bears (50 subadults, 22 adult females and 19 adult males) collected from 1999 to 2002, were multilobulated similar to *e.g.* domestic cow (*Bos taurus*) and ringed seal (*Phoca hispida*) (*e.g.* Dragert et al., 1975; Habel, 1992, Sonne-Hansen et al., 2002) and the histology of each functional unit (lobule) appeared to be similar to marine mammal renal tissue in general (*Ibid.*). It was not possible to investigate the entire kidney macroscopically during the hunt and sampling.

### Glomerular lesions

Moderate and severe uniform glomerulonephritis and –sclerosis was found in 38 of the 91 examinated East Greenland polar bears (Fig. 1). When these lesions were present, PAS-positive (hyalinisation) increase of the mesangial matrix was found (Fig. 1). In the examination of the basement membranes by PAS-M silverstaining, no humps (segmental thickening) were present but we could detect obvious mesangial cell-proliferations, but no proliferations of the glomerular endothelial cells, while occasionally proliferation of visceral epithelial cells of Bowman's capsule was found. In few cases small glomerular unifocal hyalin nodules were found as well as segmental capsular adhesions between glomerular basement membranes and Bowman's capsule with luminal PAS-positive hyalin depositioning of Bowman's space. Dense PAS-positive total hyalinisation (sclerosis) of the glomeruli was found in the most severe cases of glomerulonephritis – and sclerosis and tubular atrophy and fibrosis (Fig. 2).

**Figure 1**
**Bottom:** example of glomerular changes in a 22-year-old East Greenland female polar bears sampled in August 1999 (group: moderate). Arrows indicate thickening of the glomerular basement membrane and segmental capsular adhesion between glomerular basement membranes and Bowman's capsule with luminal PAS-positive hyalin depositioning (PAS, 40x). Bar: 50 μm. **Top:** example of glomerular changes in a 19-year-old East Greenland female polar bear sampled in January 2000 (group: severe). Arrows indicate thickening of the glomerular basement membrane and an increase in the mesangial matrix (PAS, 40x). Bar: 50 μm.





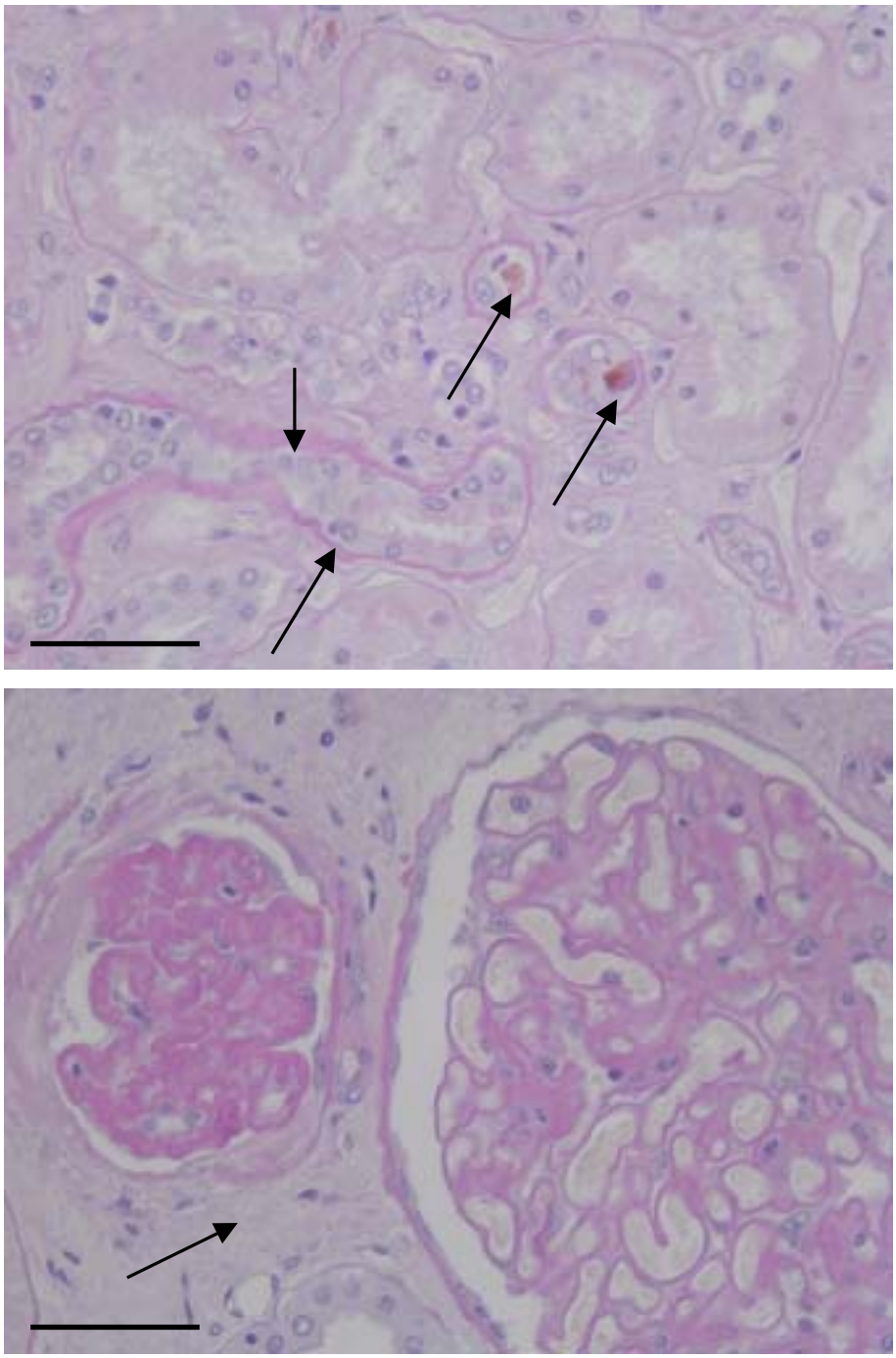


**Figure 2**
Renal lesions in a 19-year-old female polar bear sampled in January 2000 (same as Fig. 1). **Top:** obvious hyalinisation of tubular basement membranes (PAS, 20x). Bar: 25 μm. Arrows indicate hyalinisation of tubular basement membranes and macrophages containing PAS-positive debris within atrophic tubules. Note the large amounts of fibrous tissue around the hyalinised basement membranes. **Bottom:** total glomerular sclerosis (left) (PAS, 40x). Note the large amount of fibrous tissue around the glomerulus (arrow). Bar: 50 μm.

### Tubular lesions

Tubular lesions were PAS-positive hyalinisation of the tubular basement membrane with tubular atrophy and fibrosis (Fig. 2). The lesions were in severe cases accompanied by total glomerular hyalinisation (sclerosis) (Fig. 2). Furthermore, hyperplastic cell-proliferation, within the basement membrane of a distal convoluted tubule, was found in a 7-year-old male polar bear (Fig. 3). The cells were large and pale with a polygonal and monomorphic appearance prominating into the luminal area. Finally, tubular intracellular protein droplets and pigments – localised basally and apically – was found in the entire nephron of all individuals (Fig 3). The pigments were tested negative for both lipofuscin and haemosiderin and could therefore be bile pigments, haemoglobin, melanin or byproducts from the metabolism of plant material during summer (Lyon et al., 1991).

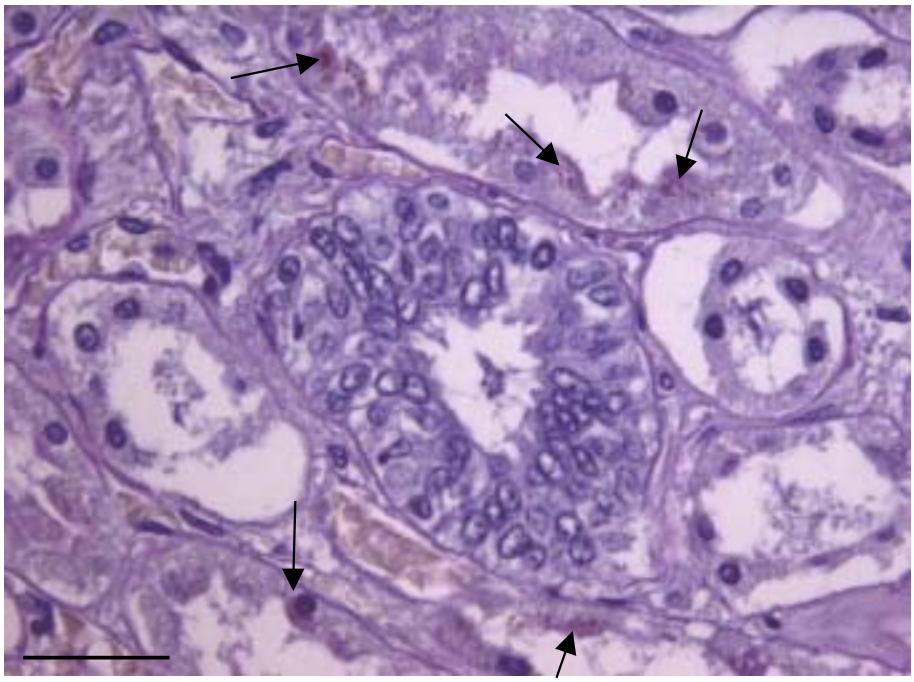


**Figure 3**
Tubular cell proliferation at the cortico-medullary border of a 7-year-old male East Greenland polar bear sampled in January 2001 (PAS, 40x). Arrows indicate pigments in distal convoluted tubules. Note the pale colour and the polygonal shape of the cells. Bar: 50 µm.

## Interstitial changes

Mononuclear cell infiltrations (lymphocytes, plasma cells and macrophages) were found in cortex and papilla in 35 individual of varying degrees (Fig. 4), and was categorised as chronic focal non-suppurative interstitial nephritis. In 25 of these cases, the infiltrates were located in cortex and/or medulla and in 10 cases located in the papilla (one 6-year-old male exhibited both cortical, medullary and papillary interstitial nephritis). In cases of intense cell infiltrations these were accompanied by tubular atrophy (Fig. 4), but rarely fibrosis or medullary luminal hyaline casts of protein cylindres.

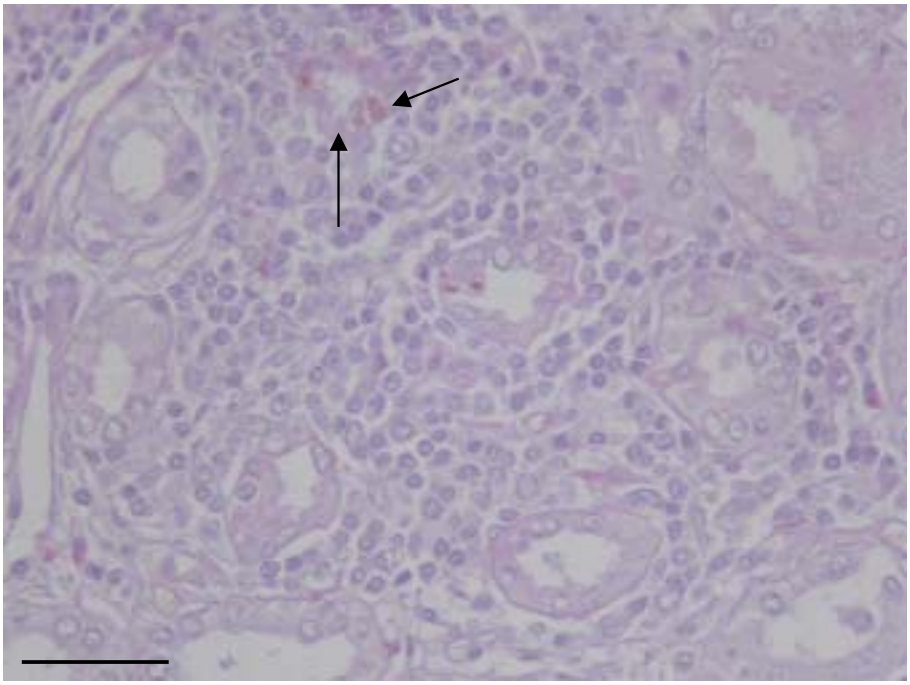


**Figure 4**
Intense focal mononuclear cell infiltration of interstitial renal tissue of a 3.5-year-old female East Greenland polar bear sampled in September 1999. Arrows indicate atrophic tubule (with macrophages containing pigments) (PAS, 20x). Bar: 25 µm.

**Table 1**
Classification of 91 East Greenland polar bears sampled during 1999-2002, into three groups of renal lesions (mild, moderate and severe) based on glomerular/tubular lesions, hyalin casts, tubular protein droplets and pigments and interstitial changes. Presence of histopathological changes is given by "-": absent, "(+)": mild, "+": moderate and "++": severe.

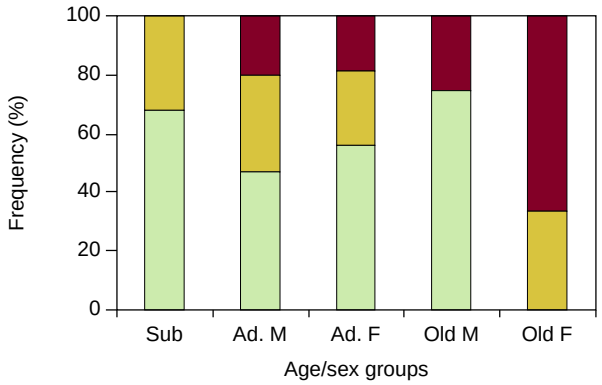
| | Mild (n=53) | Moderate (n=27) | Severe (n=11) |
|---|---|---|---|
| Uniform glomerulonephhritis and -sclerosis | – | + | ++ |
| Hyalinisation of tubular basement membrane accompanied with atrophy and fibrosis as well as total glomerular sclerosis | – | + | ++ |
| Tubular cellproliferation | – | – | (+) |
| Medullary cylindric hyaline casts | (+) | + | ++ |
| Tubular protein droplets and pigments | + | + | + |
| Interstitial mononuclear cell infiltrations | (+) | (+) | (+) |

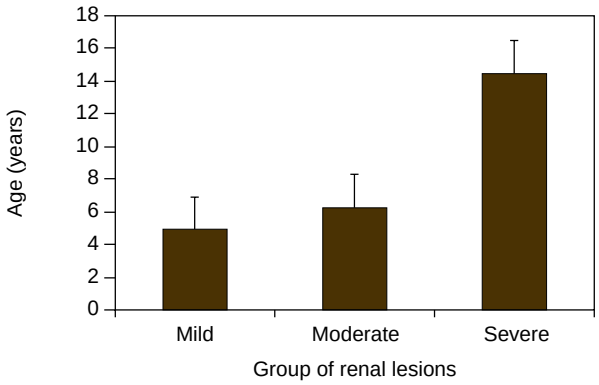
## Categorisation

To test the relationship between histopathological changes, age, sex and season, all individuals were categorised into one of three groups of severity (mild, moderate and severe), based on the glomerular, tubular and interstitial lesions (Table 1). This categorisation was also used in the further data-handling of histopathological changes in relation to age/sex, season and contaminants.

## Relationships between renal lesions, age and sex

Fig. 5 shows that no subadults exhibited severe renal lesions, while the prevalence of mild, moderate and severe lesions, respectively, was the same in adult females and adult males ($p$>0.6). This was also reflected in the mean age of bears with severe lesions, which was significantly higher compared to bears with mild and moderate lesions, respectively (both: $p$<0.05) (Fig. 5). There were indications of old females having higher frequencies of moderate and severe changes when compared to old males (Fig. 5), however, this could not be concluded due to the relatively low sample size.




| | Sub | Ad. M | Ad. F | Old M | Old F |
|---|---|---|---|---|---|
| Severe | 0 | 3 | 3 | 1 | 4 |
| Moderate | 16 | 5 | 4 | 0 | 2 |
| Mild | 34 | 7 | 9 | 3 | 0 |

**Figure 5**
**Left:** Prevalence (%) of renal lesions in 91 East Greenland polar bears sampled from 1999 to 2002. The lesions are divided by degree of severity (mild, moderate and severe) and age/sex (subadults, adult females and adult males). No. of observations are given in the table. **Right:** Mean age (years) of the individuals categorised into mild, moderate and severe renal lesions. SD shown at the top. Adult males: ≥6 years, adult females ≥ 5 years, old males and females: ≥ 15 years and others as subadults.

### Hyalin casts

Hyalin casts were evaluated in only 77 of the 91 individuals examined due to autolysis and / or freeze damage. Large amounts of tubular hyaline casts (cylindres) were found in 71% (24 of 34) of the individuals categorised into groups of moderate / severe changes and in 37% (16 of 43) of the individuals categorised into groups of mild changes (Table 1). This difference was significant ($p<0.01$) indicating that tubular protein loss is increasing with increasing renal lesions. There were no significant relations between interstitial changes (cellinfiltrations) and hyaline casts, nor a relation between glomerular / tubular changes and interstitial changes (both: $p>0.11$).

### Interstitial mononuclear cellinfiltrations

No significant relation was found between interstitial cellinfiltrations and medullary luminal hyaline casts ($p>0.1$) nor between interstitial cellinfiltrations and glomerular / tubular lesions ($p>0.28$). Beside this, the presence of renal interstitial cellinfiltrates within each individual were significantly related to the degree of mononuclear cell infiltrates in the liver parenchyma (granulomas) or portal areas ($p<0.05$) (Sonne et al., Submitted).

### Seasonal variations in renal lesions

No seasonal difference, in the severity (mild, moderate and severe) of renal lesions, was found between period of hibernation (Oct-Mar) and period without hibernation (Apr-Sept) ($p>0.19$).

### Relationship between histopathology, contaminants and BMD

Age sex and seasonal variations in the levels of organochlorines in East Greenland polar bears have been reported previously by Dietz et al. (2004). Briefly, levels of ∑-PCBs, ∑-DDTs, dieldrin, ∑-HCHs and ∑-PBDEs did not differ by age or sex groups (subadults, adult females and adult males) (all: $p>0.12$) while levels of HCB in subadults of both sexes were significantly higher than in adult males ($p<0.01$) and ∑-CHLs were significant higher in adult females when compared to adult males ($p<0.04$).

Levels of ∑-PCBs, ∑-DDTs, ∑-CHLs, dieldrin, ∑-HCHs, HCB and ∑-PBDEs within groups of renal lesions (mild, moderate and severe) divided on age and sex (subadults, adult females and adult males, respectively) are showed in Table 2. We tested if the concentrations of each organohalogen group differed between the degree of histopathological changes (mild, moderate and severe, respectively) within the age / sex group, but no such difference could be detected (all: $p>0.08$) (the relatively small sample size in some of the groups which could have influenced the test results).

In addition, we tested whether there, for subadults and adults, was a difference in skull bone mineral density (BMD) between groups of renal lesions (mild, moderate and severe) (Table 3). We found no difference in skull BMD between histology groups for a sub-set of 48 subadults and adult females (both: $p>0.13$). However, in 12 adult males examined, BMD levels were significantly higher in the group of severe lesions when compared to the group of moderate lesions and significant higher in the group of mild lesions when compared to the group of moderate lesions (both: $p<0.05$) (Table 3). We would expect the BMD to increase in the order: severe<moderate<mild, and the difference could therefore be a result of the small sample size. Further information on age relations and the negative correlation between ∑-PCBs, ∑-DDTs, dieldrin and ∑-CHLs and skull BMD is reported in (Sonne et al., Accepted with revision).

**Table 2**
Mean±SD concentrations of organohalogens (ng/g l.w.) in subcutaneous adipose tissue of a sub-sample (*n*=79) of 91 East Greenland polar bears investigated for renal lesions during 1999-2002 (∑=sum). The concentrations are divided on age/sex groups (subadults, adult females and adult males respectively) and renal histological changes (mild, moderate and severe, respectively). No. of samples are given in paranthesis (contaminant data were not available for all 91 individuals examined histologically).

| Contaminant group | Histopathology | Subadults | Adult females | Adult males |
|---|---|---|---|---|
| | Mild | 6144±2947 (29) | 5457±1801 (8) | 8239±3992 (9) |
| ∑-PCBs | Morderate | 5690±2054 (14) | 7679±6472 (6) | 8222±4988 (2) |
| | Severe | . | 4949±2838 (7) | 7229±3264 (4) |
| | Mild | 375±187 (29) | 419±237 (8) | 424±200 (9) |
| ∑-DDTs | Morderate | 512±219 (14) | 387±100 (6) | 501±531 (2) |
| | Severe | . | 244±210 (7) | 446±473 (4) |
| | Mild | 1453±925 (29) | 1398±440 (8) | 978±615 (9) |
| ∑-CHLs | Morderate | 1423±722 (14) | 2430±2559 (6) | 1172±627 (2) |
| | Severe | . | 1252±657 (7) | 810±257 (4) |
| | Mild | 195±103 (29) | 168±45 (8) | 149±83 (9) |
| Dieldrin | Morderate | 210±60 (14) | 221±79 (6) | 214±143 (2) |
| | Severe | . | 155±77 (7) | 139±94 (4) |
| | Mild | 187±69 (29) | 171±34 (8) | 246±185 (9) |
| ∑-HCHs | Morderate | 178±66 (14) | 162±72 (6) | 234±39 (2) |
| | Severe | . | 206±276 (7) | 233±190 (4) |
| | Mild | 91±85 (29) | 76±68 (8) | 45±31 (9) |
| HCB | Morderate | 104±67 (14) | 85±112 (6) | 29±37 (2) |
| | Severe | . | 58±56 (7) | 44±21 (4) |
| | Mild | 55±30 (30) | 63±32 (8) | 48±20 (9) |
| ∑-PBDEs | Morderate | 54±34 (14) | 57±15 (6) | 113±66 (2) |
| | Severe | . | 72±58 (7) | 41±18 (4) |

**Table 3**
Mean (SD) levels of Bone Mineral Density (BMD; g hydroxyapatite/cm$^2$) in the skulls of 60 East Greenland polar bears investigated for kidney lesions during 1999-2002. The concentrations are divided on age/sex groups (subadults, adult females and adult males) and groups of renal lesions (mild, moderate and severe). No. of samples are given in paranthesis. "*": indicate statistical significant differences in BMD levels between groups of mild and moderate lesions at the 1% level. "‡": indicate statistical significant differences in BMD levels between groups of moderate and severe lesions in adult males at the 1% level. "n.s." indicate no statistical significant differece between any of the histopathological groups.

| Histopathology | Subadults $^{n.s.}$ | Adult females $^{n.s.}$ | Adult males $^{*, ‡}$ |
|---|---|---|---|
| Mild | 1.7±0.3 (24) | 2±0.1 (6) | 2.5±0.2 (6) |
| Moderate | 1.8±0.3 (10) | 2±0.1 (4) | 1.9±0.1 (3) |
| Severe | . | 1.9±0.2 (5) | 2.6±0.3 (3) |

## Adrenals

None of the 43 polar bears exhibited macroscopic adrenal pathological changes. The histological appearance of the polar bear adrenals was similar to that in other carnivores in general. No histopathological changes was found, but 7 of the examined bears exhibited cortical hyperemia, probably due to stress from the hunt (Fig. 6).

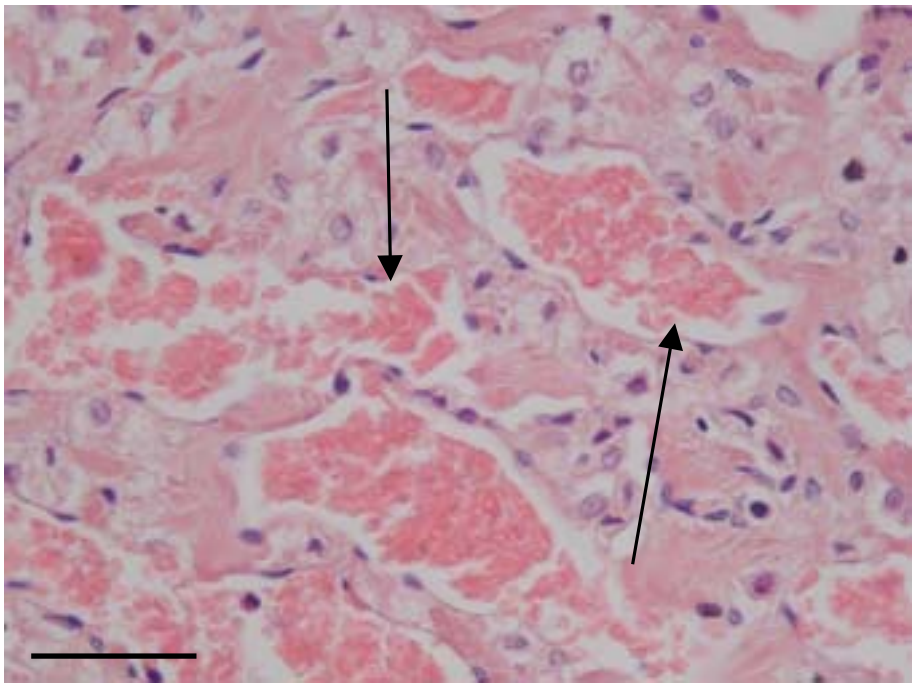


**Figure 6** Hyperemia (arrows) in the adrenal cortex of a 6.5-year-old male East Greenland polar bear sampled in April 2001 (HE, 40x). Bar: 50 µm.

## Discussion

### Kidney

#### Glomerular and tubular lesions

PAS-positive proliferation (often immune complex mediated) of the glomerular basement membranes (membranous glomerulonephritis) and mesangium, as those found in the polar bears, is a common sequela in marine mammals (*e.g.* Bergman and Olsson, 1985; Bergman et al., 2001; Sonne-Hansen et al., 2002; Woshner et al., 2002), and is also found in domestic mammals and humans (*e.g.* WHO, 1992, Beirne et al., 1972; Zimmerman et al., 1975; Maxie et al., 1993; Churg et al., 1995; Confer and Panciera, 1995; Cotran et al., 1999). These immuno reactions have been associated with exposure to toxic substances (organohalogens, heavy metals and organic solvents) as well as virus and bacteria (*e.g.* Bergman and Olsson, 1985; Bergman et al., 2001; WHO, 1992; Beirne and Brennan, 1972; Zimmerman et al., 1975; Maxie et al., 1993; Churg et al., 1995; Confer and Panciera, 1995; Cotran et al., 1999).

PCBs and DDTs are known xenoestrogens (*e.g.* de March et al., 1998) and prolonged exposure to these have in rats produced hyperplasia of papillary epithelia (Bruckner et al., 1974a). Prolonged exposure of rodents, pigs and domestic dog to estrogens (*i.e.* stilbestrol) produced increased mesangial width and celluarity, increased amounts of interstitial fibrous tissue with atrophy of glomeruli and tubuli as well as and squamous metaplasia of urogenital tract epithelia (collecting ducts and distal tubules) (Horning et al., 1954; Trevan, 1956; Matthews et al., 1947, Zayed et al., 1998). Although the metaplasia in these studies were found in the very distal parts of the nephrons, the 7-year-old East Greenland male polar bear exhibing epithelial hyperplasia of the distal tubule could indicate environmental exposure to xenoestrogens (*i.e.* PCBs, DDTs etc.) and not being age related. In addition to this, we also found increased mesangial width, interstitial fibrosis and glomerular / tubular atrophy in groups of moderate and severe renal lesions, in the polar bears.

Effects of firemaster BP6 – a PolyBrominated Biphenyls - was investigated in dogs and rats (McCormack et al., 1978). The overall histological changes detected, were shrunken glomeruli and a single case of mononuclear cell infiltration, which both were found in several cases in the present East Greenland polar bears. Beside this, the arylhydrocarbon hydroxylase (AHH) increased in exposed rats compared to the controls which indicated a biochemical effect of the contaminants on renal tissue. Grinwis et al. (2001) showed that European flounders (*Platichthys flesus*) exposed to PCB-126 exhibited immunoreactivity in the proximal tubules of the mesonephros and an increase of stationary / circulating mononuclear cells. Although the exposure dose in the flounders was significantly higher, compared to East Greenland polar bears, the study by Grinwis et al. (2001) showed that renal tissue (tubules) is affected by some coplanare PCB-congeners.

We speculated whether the tubular protein droplets and pigment accumulation in the polar bears could be related to the mesangial and glomerular damage or to the food composition (*e.g.* plant material), season (fasting, hibernation), age, infections or maybe PCB exposure (Bruckner et al. 1974a, b; Confer and Panciera 1995, MacLachlan and Cullen 1995, Bergman et al. 2001). However, this could not be investigated further. Whether the increased protein loss to the tubular lumen (hyalin cylindres) in individuals with moderate to severe renal lesion, clinically affected these, could not be evaluated. It must also be considered whether the tubular droplets have a link to e.g. mercury exposure as described in free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) (Rawson *et al.* 1993) and Arctic beluga whales (*Delphinapterus leucas*) (Woshner *et al.* 2002).

### Interstitial changes

The interstitial nephritis found in the present study were similar to those found in ringed seals from North West Greenland (municipality of Thule) (Sonne-Hansen et al., 2002) and Baltic seals (Bergman and Olsson, 1985; Bergman et al., 2001). These interstitial changes found in the present polar bears were thought to be a result of infections and / or immunological reactions from injury of blood vessels and tubules (*e.g.* Confer and Panciera, 1995).

### Age, sex and seasonal changes

The age / sex differences with adults and old bears carrying higher grades of renal lesions than subadults were in accordance with the findings by Bergman et al. (2001) who found that such lesions in Baltic seals, among other things, was due to old age. The indications of old females exhibiting higher frequencies of moderate and severe changes, compared to old males, may indicate that females are more susceptible to extrinsic environmental factors (*e.g.* infections, organohalogens a.o.) than males, due to gestation and nursing, where large amounts of organohalogens are released into the blood stream and thereby become biologically active in the target organs (*e.g.* Polischuk et al., 1995, 2002).

Histologic changes in the kidneys have been reported in Romanian brown bear (*U. arctos*) (Prunescu et al., 2003). A seasonal variation in the PAS-positive deposits of the mesangium (low in autumn and high in spring), pericapillary fibrosis of the glomeruli as well as a large space between the corpuscle and Bowman's capsule were seen, and it was thought that these changes should be pre-hibernation physiological adaptations (Prunescu et

al., 2003). We did not find such a seasonal difference in polar bears probably due to the fact that only pregnant polar bears den for a long time (we did not have samples from pregnant polar bears) whereas all other polar bears of both sexes and age only periodically use shelters (faculative dens) when environmental conditions are adverse (*e.g.* Ferguson et al, 2000).

### Histological changes in relation to contaminants and BMD

The reason for lack of correlations between organohalogen levels and renal lesions, could be the relatively large individual variability due to age, sex, season and genetics, which make the adipose tissue concentration of organohalogens a poor measure of the individual life long exposure. In addition, female transference of organohalogens to the offspring reduces the general body burden which again reduces the value of using body concentration as a measure of exposure for females (*e.g.* Polischuk et al, 1995, 2002). Total exposure which, at least for some effects, is of importance cannot be detected from body concentrations at late stages of life. The indications of relations between bone density and renal lesions in adult males could indicate a dysfunction in the glomerular and tubular reabsorption mechanisms as indicated by the tubular protein loss (Friberg et al., 1986; WHO, 1992; Domrongkitchaiporn et al., 2002). However, that BMD in the group of severe changes should be higher than in the group of mild lesions doesn't make sense, and it could therefore be a result of the relatively small sample size.

It was not possible to evaluate whether the possible organohalogen induced lesions of pluripotent Ito-cells and hepatocytes in the liver tissue of the present polar bears (Sonne et al., submitted) have contributed to the renal lesions through a vitamin A release with subsequent toxicity to renal tissue (*e.g.* Sato et al., 2001).

### Pollution as a co-factor?

The histopathological changes found in glomeruli, tubuli and interstium were to som degree the same as those reported in Baltic grey seal (*Halichoerus grypus*) and ringed seal heavily exposed to PCBs, DDTs and heavy metals between 1977-1996 (Bergman and Olsson, 1985; Bergman et al., 2001). These studies suggested the lesions to be a result of age, but based on a low-exposed reference material from Svalbard, their investigation also indicated chronic exposure to organohalogens as a plausible etiological factor. Also, environmentally PCB contaminated bream-fish (*Abramis brama*) and asp-fish (*Aspius aspius*) in a lake in Southern Finland exhibited dilatation of glomerular capillaries, mesangial edema and adhesions between visceral and parietal layers of Bowman's capsule with filling of Bowman's space (Koponen et al., 2001). These changes were more or less the same as those found in the present polar bears.

The renal lesions in the present East Greenland polar bears were all similar to the histopathological changes in PCB exposed laboratory animals, wild Baltic seals and wild fish. In addition to this, severe lesions were only found in adult and old animals, which propose age as the reason for this. But, the PCB levels in East Greenland polar bears are supposed to have been significantly higher around 1985 (*e.g.* Dietz et al., 2004), and therefore organohalogen exposure could be a co-factor in the development of these present lesions in adult and old animals. We therefore suggest that age and exposure to organohalogens are the two major factors in the pathogenesis of the renal lesions found in the East Greenland polar bears.

### Adrenals

Several studies have dealt with adrenolytic processes induced by specific DDT metabolites (*e.g. 4,4'*-DDD) causing necrosis of the *zona fasciculata* (*e.g.* Brandt et al, 1992). Bergman and Olsson (1985) reported a generalised stress syndrome in Baltic grey and ringed seals with adrenocortical hyperplasia (Cushing's syndrome) probably related to organohalogens within the period 1977-1996. In polar bears, Oskam et al. (2004) have documented disruption of cortisol homeostasis due to PCB and pesticide contaminants (*n*=251). In the East Greenland polar bears reported here, we could not find evidence of contaminant induced general or nodular hyperplasia nor necrosis in any of the adrenals examined, probably due to a sub-effect exposure to organohalogens.

## Conclusions

The glomerular and tubular renal lesions found in the present East Greenland polar bears were to some degree similar to those found in highly PCB and DDT polluted seal populations in the Baltic, and in PCB exposed laboratory animals. The lesions were age related and sex differences in old animals were indicated (higher prevalence of renal lesions within groups of adult/old females compared to adult/old males). A weak relationship between bone mineral density and renal lesions in adult males could indicate glomerular and tubular dysfunctions, or it could be a result of low sample size. No lesions were found in adrenals. We therefore suggest that the renal lesions found in the polar bears were a result of ageing and infections but long-term exposure to nephrotoxic heavy metals (Cd, Hg) and organohalogen compounds cannot be ruled out as co-factors.

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# Paper VI
# Enlarged clitoris in wild polar bears (*Ursus maritimus*) can be misdiagnosed as pseudohermaphroditism.

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## Abstract

A 23-year-old female polar bear (*Ursus maritimus*) killed in an Inuit hunt in East Greenland on July 9, 1999 had a significantly enlarged clitoris resembling, in size, form and colour, those of previously reported 'pseudohermaphroditic' polar bears from Svalbard. It has been suggested that an enzym defect (21-hydroxylase deficiency), androgen producing tumour or high exposure to organochlorines during the foetal stage or early development could be the reason for the supposed pseudohermaphroditism observed for Svalbard bears. Except for the enlarged clitoris, all dimensions of the external and internal reproductive organs of this bear were similar to a reference group of 23 normal adult female polar bears from East Greenland collected in 1999-2002. The aberrant bear was a female genotype, and macroscopic examination of her internal reproductive organs indicated that she was reproductively functional. A histological examination of the clitoral enlargement in the present East Greenland specimen allows a first-time histological evaluation of the earlier macroscopic field diagnosis from Svalbard. This examination revealed intense chronic ulcerative and perivascular *clitoriditis* similar to "acral lick dermatitis" frequently seen in domestic dogs (*i.e.* we did not find any signs of pseudohermaphroditic hyperplasia due to androgenic or antiestrogenic endocrine disruption). The levels of organohalogens and TEQ values were lower than concentration thresholds of toxicological risk. It is hence possible that the previously reported adult female polar bear pseudohermaphrodites from Svalbard are in fact misdiagnoses. Therefore, future studies examing pseudohermaphrodism in wildlife should consider that certain occurrences are natural events, *e.g.*, enlarged clitoris in the present East Greenland polar bear. Furthermore, caution should be exercised in suggesting linkages of such inflammatory abnormalities with correlations to anthropogenic pollutant exposures.

*Key words:* polar bear, *Ursus maritimus*, POP, DDTs, PCBs, PBDEs, HCHs, CHLs, furanes, dioxin, stress, pseudohermaphroditism, misdiagnosis, histopathology.

## Introduction

Polar bears (*Ursus maritimus*) from the eastern Atlantic Arctic including East Greenland have higher levels of persistent organochlorine (OC) contaminants such as polychlorinated biphenyls (PCBs) and *bis*-2,2-(4-chlorophenyl)-1,1,1-trichloroethane (4,4'-DDT) and its metabolites 4,4'-DDE and 4,4'-DDD, than reported in tissues of polar bears from populations elsewhere in the Arctic (Norstrom et al., 1998, Andersen et al., 2001, Lie et al., 2003). Marine ecosystems in the eastern Atlantic Arctic receive a relatively high air and seaborne input of OCs from sources in lower latitudes compared to other Arctic areas (de March et al., 1998). Levels of PCBs in polar bears from East Greenland, Svalbard (Norway) and the Kara Sea (Russia) are similar to those believed to elicit negative effects on the reproduction and survival of seals from the Baltic Region (de March et al., 1998, AMAP, 2004).

OCs released into the environment are readily absorbed in organic matter, becoming particularly bioconcentrated and subsequently bioaccumulating in waxy and fatty tissues (Norstrom and Muir 1994). Bioaccumulation and thus greater exposure to OCs may have adverse health effects on re-production, the immune and endocrine systems and metabolism in wildlife. Arctic marine mammals, and especially a carnivorous predator such as polar bear, are susceptible to high OC exposures due to their great reliance upon accumulating large lipid stores for thermoregulation and energy storage (de March et al., 1998, AMAP, 2004). Owing to the high lipid content of their diet, polar bears are particularly prone to bioaccumulation of OCs. Polar bears are at the top of the Arctic marine food chain, and prey primarily on the blubber of ringed seal *(Phoca hispida)* and to some extent on bearded seals *(Erignathus barbatus)* (Derocher et al., 2002).

OCs and/or their persistent metabolites (*e.g.*, oxychlordane, 4,4'-DDE, methyl sulfone ($MeSO_2$) PCBs and hydroxylated (HO) PCBs) have been reported in polar bear tissues, and can be transferred *in utero* and via lactation from mother to foetus and cub (Norstrom and Muir 1994, Letcher et al., 1998, Norstrom et al., 1998, Polischuk et al., 2002, Sandala et al., 2004, Sandau et al., 2000). Several of these contaminants have been shown to be agonists and/or antagonists to hormone-dependent processes, and thus potentially disrupt or modulate endocrine systems in a variety of glands and internal organs including reproductive organs in both sexes (Colborn et al., 1993, Sandau et al., 2000, Damstra et al., 2002, Letcher et al., 2000, 2002).

One conspicuous effect of hormonal disruption on reproductive organs is pseudohermaphroditism (Benirschke 1981, Polani 1981, Acland 1995, Capen 1995, Feldman 1995, Hunter 1995, Mickelsen and Memon 1995). A pseudohermaphrodite is an individual with apparently healthy gonads of only one sex but which also has some traits of the opposite sex (*Anon.* 1994). Pseudohermaphroditism in wildlife has only been reported in 11 individual mammals: 4 female polar bears (*U. maritimus*) from Svalbard (Wiig et al., 1998), 4 female black bears (*U. americanus*) and one brown bear (*U. arctos*) from Alberta (Cattet 1988) and four male bowhead whales (*Balaena mysticetus*) from Alaska (Tarpley et al., 1995, O'Hara et al. 2002). The etiology for the black and brown bears were endocrine disruption due to phytoestrogens (Cattet, 1988) while androgen producing tumour, 21-hydroxylase deficiency and organohalogens were proposed to be possible factors in the pathogenesis of the Svalbard polar bears (Wiig et al., 1998). However, as samples could not be taken out from these polar bears, it was not possible to evaluate whether these were real pseudohermaphrodites. Of these four individuals, the two

adults were supposed to be fertile while the reproductive parameters of the yearlings could of course not be evaluated.

Because hunting of polar bears is not permitted on Svalbard, and because the pseudohermafrodites seen there were caught during live-capture of polar bears, their external appearance could only be examined visually. Two adults had an enlagened clitoris and two yearlings (siblings) had enlarged clitoris and laterally situated urethral opening. It was suggested that the enlarged clitoris were congenital and could have been caused by an enzym defect (21-hydroxylase deficiency), androgen producing tumour or a high exposure to organochlorines during the foetal stage or early development of the reproductive organs (Wiig et al., 1998).

The present study examines physiological and chemical exposure factors that may provide clues to the cause(s) of the enlargement of the clitoris that has been observed in wild polar bears. The reproductive organs of an female polar bear with enlarged clitoris from East Greenland (1999) are described on the basis of macro- and microscopical examinations. A likely explanation of the enlargement of the clitoris is given based on the histological analyses.

## Materials and methods

### The sampling

All samples were taken <12 *h post mortem* and preserved during the subsistence hunt by Inuit in East Greenland, 1999-2002. Samples of adipose tissue (1999-2002) were stored in separate PE plastic bags until arrival in the laboratory, where they were transferred into rinsed glass containers with lids of aluminium. Samples of genitalia (entire reproductive tract including external genitalia), kidney, liver, spleen, adrenals and lymph nodes were stored in a combination of formaldehyde and alcohol (10% of a 35% formaldehyde solution and 90% of a 96% ethanol solution). All samples were kept at minus 20 ºC before examination in the laboratory in Copenhagen. The reproductive tracts of 23 adult female polar bears (ages: 5-25 years) that had been collected during 1999-2002 in the Scoresby Sound area served as reference material. These tracts had also been collected by local hunters and treated as described above.

### Age estimation

Individual ages were estimated by counting the growth layer groups in the cementum of incisors ($I_3$) after decalcification, thin sectioning (14µm) and staining with toluidine blue (Hensel and Sorensen 1980, Dietz et al., 1991).

### Genetic analyses

DNA was extracted according to Andersen et al., (1998). For sex determination of the polar bear, primers for the SRY (sex-determining region Y-gene) and ZFY / ZFX (Y-linked and X-linked zinc finger genes, respectively) genes were used (Amstrup et al., 1993, Taberlet et al., 1993) as the SRY and ZFY fragment sequences are found only in male polar bears and not in females (Amstrup et al., 1993).

### Macroscopic examination of reproductive organs

In the field, the hunter made a superficial macroscopic examination before fixing the bear´s external genitalia in formalin. In the laboratory the genitalia were examined visually and then dissected and inspected macroscopically. The various organs were measured to the nearest mm, and the ovaries and uterine horns were cut open for examination of follicles, corpora lutea and/or albicantia, unimplanted blastocysts and placental scars (Rosing-Asvid et al., 2002).

### Histology

All tissue samples were trimmed, processed conventionally, embedded in paraffin, sectioned at about 4 µm and routinely stained with haematoxylin-eosin (HE) (Lyon et al., 1991). All slides were examined on a Leica DMLB microscope with 50, 100, 200, 400, 630 and 1000 x magnification and pictures were digitised using a Nikon Coolpix 5000 camera.

### X-ray (osteodensitometry)

The X-ray osteodensitometry was applied to detect osteopenia (osteoporosis) by use of a Norland XR 26 X-ray bone densitometer determining the bone mineral density (calcium-phosphate/$cm^2$) through a dual X-ray absorptiometry (DXA), where a high stable X-ray tube generates a broad spectrum of photons k-edge filtrated into two distinct peaks (The Norland Corporation 1993). The skulls were scanned in "Research" (speed: 60mmx60mm; resolution: 3.0x3.0; width: 24,9cm) and analysed in XR software revision 2.4®, which generated a picture of the bone segment and calculated the bone mineral density (BMD; gram/$cm^2$) (*Ibid.*).

To ensure that the BMD in skull represents the mineral status of the body skeletal system in general, a pilot study was conducted where the BMD of skull, femur and three lumbale vertebrae was compared in a total of 13 polar bears from Copenhagen Zoo and East Greenland. The analysis showed a highly significant correlation between BMD in skull and femur ($r$ = 0.99; $p$<0.001; $n$=13), and skull and vertebrae ($r$ = 0.97; $p$<0.001; $n$=9). These results justified the use of BMD in skull to reflect the BMD status of the entire skeletal system. The DXA-scanner was daily calibrated using a phantom with known mineral density (demands: precision > 99%; accuracy between ±2SD). In addition the precision was tested by a 10 time rescanning giving 99.88% ((1 – (std.dev./mean)) x 100%) = (1- (0.6041/ 521.96)) x 100% = 99.88%).

Fragmentation of teeth caused by handling was thought to be a problem. A double determination of the BMD in 2 skulls (#5483 and #2891) with and without insicors, canines, premolars and molars showed that only loss of half or more of the canines altered the result significantly. As no canines were fragmentet half or more; this was not considered a problem.

### Contaminant analyses

#### PCBs and OCs

Polar bear adipose tissue samples were analysed according to Sandala et al., (2004) and Dietz et al., (2004) at the Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada. An external standard

quantification approach used for PCBs and OCs in the adipose tissues was based on peak area of the GC-μECD response, which is described in detail in Dietz et al., (2004). Briefly, PCBs is the sum (Σ) of the concentrations of the 51 individual or co-eluting congeners (if detected): PCB # 31/28, 52, 49, 44, 42, 64/71, 74, 70, 66/95, 60, 101/84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129/178, 182/187, 183, 128, 174, 177, 171/ 202/156, 200, 172, 180, 170/190, 201, 203/196, 195, 194, 206. Σ-DDTs is the sum of 4,4'-DDT, 4,4'-DDD and 4,4'-DDE. Σ-HCHs is the sum of the α-, β- and λ-hexachlorocyclohexane. Σ-CHLs is the sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. Contaminant fractions were subsequently sent to the National Water Research Institute (Environment Canada, Burlington, Ontario, Canada L7R 4A6 (NWRI)) for determination of brominated diphenyl ether (PBDE) flame retardants.

#### PBDEs, non-ortho PCBs and PCDD/Fs

BDPEs were determined by electron capture negative ion (low resolution) MS using an external standard. Briefly, PBDEs is the sum (Σ) of the concentrations of the 35 individual or co-eluting congeners (if detected): PBDE# 10, 7, 11, 8, 12/13, 15, 30, 32, 28/33, 35, 37, 75, 71, 66, 47, 49, 77, 100, 119, 99, 116, 85, 155/126, 105, 154, 153, 140, 138, 166, 183, 181, 190. Gas chromatographic conditions for the PBDEs were described by Luross et al. (2002).

Extraction and analysis of PCDD/Fs in polar bear fat was conducted by Axys Analytical Services (Sidney, BC, Canada) used previously described techniques with only minor modification (U.S. EPA, 1998, 1999). Fat samples were homogenized with Na2SO4 and spiked with mixture of 13C-labelled non-ortho PCBs, as well as PCDDs (2,3,7,8-TCDD, 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,6,7,8-hexaCDD, 1,2,3,7,8,9-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and OCDD) and PCDF isomers (2,3,7,8-TCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,7,8,9-hexaCDF, 2,3,4,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8,9-heptaCDF, and OCDF) surrogate standards to monitor extraction efficiency. Samples were extracted in toluene via Soxhlet and concentrated. Extracts were cleaned-up and PCDD/Fs were isolated through a series of chromatographic columns (U.S. EPA 1998). Analysis was performed using a high-resolution mass spectrometer coupled to a high-resolution gas chromatograph (HRGC-HRMS) equipped with a DB?5 capillary chromatography column (60 m ´ 0.25 mm i.d. ´ 0.1 μm film thickness; J&W Scientific, Folsom, CA, USA). A second column, DB-225 (30 m ´ 0.25 mm i.d. ´ 0.15 μm film thickness), was used for confirmation of 2,3,7,8-tetraCDF. All analytical procedures were carried out according to protocols as described U.S. EPA (1998).

### Statistical analyses

Excel 97® was employed as spreadsheet and SAS (1990) in calculations of means and confidence intervals of reproductive organ measurements and BMD. A one-tailed one-sample *t*-test was used to determine the significance ($p<0.05$) for the sizes of clitorises. A two-tailed one-sample *t*-test was used to determine the significance ($p<0.05$) of difference in size of internal reproductive organs (uterus, ovaries, follicles) and concentrations of organohalogen compounds (OHCs).

## Results and discussion

### The female polar bear

On July 9, 1999, a 23-year-old female polar bear with enlarged clitoris was shot by subsistence hunters at the entrance to Scoresby Sound (c. 70°N, 21°W) in East Greenland. Phenotypically the bear was a female that had an enlargened clitoris that appeared swollen and red. The absence of the SRY and ZFY bands characteristic of males and the presence of at least one ZFX band indicated that genetically the polar bear was a female.

### Macroscopic analyses of the reproductive organs

Anatomical examination of the abnormal polar bear's external and internal reproductive organs revealed that vulva, vagina, uterus, uterine ducts and ovaries were morphologically normal. However, by being 27mm long, 21mm high and 22mm wide at base the clitoris was significantly larger (all: $p<0.01$) than those in the reference group (Fig. 1 and Table 1).

**Table 1**
Macroscopic anatomy and basic statistics of the reproductive organs from the 23-year-old female polar bear with enlarged clitoris, compared to the reference group.

| Examined tissue | Female with enlarged clitoris | Reference group | | |
|---|---|---|---|---|
| | | Mean ± $t_{0.95}$ * SD | Min-max | *N* |
| Clitoris_length (mm) | 27* | 6.5±5.5 | 5-8 | 4 |
| Clitoris_width (mm) | 22* | 3.8±1.6 | 3-4 | 4 |
| Clitoris_height (mm) | 21* | 4±2.61 | 3-5 | 4 |
| Uterus_diameter (mm) | 22* | 7.4±5.1 | 4-14 | 17 |
| Cervix-uterus_length (mm) | 95 | 92.5±77.2 | 60-180 | 10 |
| Cornu uteri_diameter_left (mm) | 12* | 6.6±3.54 | 4-10 | 20 |
| Cornu uteri_length_left (mm) | 77 | 118.7±42.91 | 78-152 | 23 |
| Cornu uteri_diameter_right (mm) | 15* | 6.7±3.6 | 4-10 | 19 |
| Cornu uteri_length_right (mm) | 83 | 120.6±52.4 | 78-160 | 19 |
| Ovary_length_right (mm) | 23 | 22.8±9.54 | 10-28 | 21 |
| Ovary_length_left (mm) | 26 | 23.2±5.4 | 13-34 | 22 |
| Ovary_ width _left (mm) | 11 | 14.2±6 | 10-21 | 22 |
| Ovary_height_left (mm) | 16 | 16.5±12.2 | 11-23 | 22 |
| Ovary_weight_left (g) | 3.8 | 3.05±23.7 | 1.4-5.2 | 16 |
| Follicle_<1mm_right (counts) | 26 | 27.5±35 | 2-58 | 18 |
| Follicle_1-2mm_right (counts) | 1 | 5.8±9.3 | 2-14 | 11 |
| Follicle_<1mm_left (counts) | 13 | 28.6±23.6 | 10-55 | 18 |
| Follicle_1-2 mm_left (counts) | 5 | 8.2±22.9 | 1-43 | 15 |
| Follicle_2-3 mm_left (counts) | 3 | 7.3±12 | 1-15 | 8 |

*: measure not included in the 95% confidence interval of the observations.


**Figure1**
The external genitalia from the 23-year-old female polar bear with enlarged clitoris shot in Scoresby Sound, Central East Greenland 1999. **Left:** The genitalia before fixation (note angiogenesis). **Middle:** Formaldehyde-alcohol-preserved external genitalia before sectioning. **Right:** cross-section. **E:** enlarged clitoris; **Bar**: cm.

The internal reproductive organs of the aberrant polar bear did not differ significantly in size from the organs in the reference material, although the diameter of the uterus was significantly larger than that of the reference group, probably due to random biological variation or gestation (Table 1). The bear appeared reproductively healthy and follicles were present in both ovaries (average of 23 follicles; ø:1-3mm). Furthermore, one of the ovaries had a morphologically normal *corpus luteum* indicating that she had mated during spring and perhaps was in gestation. Due to the fixation in alcohol-formaldehyde it was no possible to investigate the presence of implantation scars from previous gestations. Based on the above findings, the polar bear was diagnosed as a functionally normal adult female.

### Histopathology

The polar bear's clitoris, *fossa clitoridis*, vaginal opening, uterine horns, adrenals, liver, kidney, spleen and lymph nodes (axillary and inguinal) were examined histologically. The clitoris showed loss of epithelial structures due to ulceration of the glans. The ulcer was partly covered by serocellular crusts and encompassed most of the clitoris. The surrounding non-ulcerated epithelium was hyperplastic with prominent rete ridges.

Superficially neutrophils and macrophages were the predominant inflammatory cells and melanin-containing macrophages were localized subepithelially in the area of reepithelialization. In the deeper structures the inflammatory reaction was mainly perivascular and dominated by plasma cells and lymphocytes (Fig. 2).

There was severe fibrosis of the subepithelial tissues with large numbers of capillaries orientated perpendicular to the surface of the clitoris. Some of the capillaries were thrombosed and contained large numbers of neutrophils (Fig. 2). The inflammatory reaction and vascular proliferation were not restricted to the area of ulceration, but was also found in the subepithelial tissue of the vestibule and *fossa clitoridis*. Unfortunately all cellular structures in the central part of the clitoris were lost due to inadequate fixation and freezing damage, leaving nothing but collagenous fibres.

The lesions in the clitoris were characterized as chronic-active ulcerative, proliferative and perivascular clitoriditis. The enlargement of the clitoris can be attributed to extensive accumulation of granulated tissue. The lesions resembled so-called "acral lick dermatitis", frequently seen in dogs (Yager

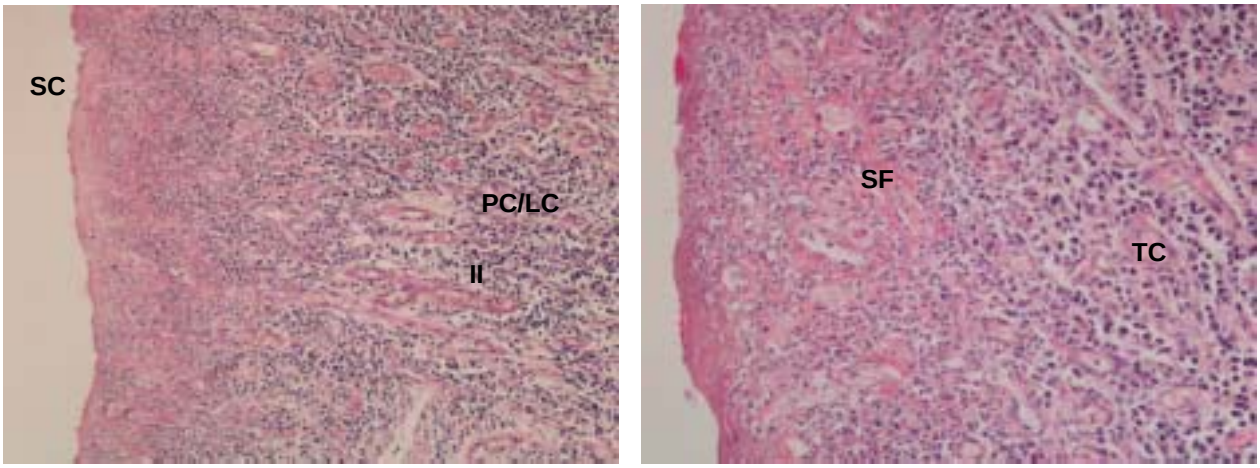


**Figure 2**
The histology of the enlarged clitoris from the 23-year-old female polar bear shot in Scoresby Sound, central East Greenland, 1999. The ulcerated part of glans clitoridis is covered by serocellular crust (SC). The tissue is highly vascularized and there is intense inflammation (II) dominated by plasma cells (PC) and lymphocytes (LC), severe fibrosis (SF) and thrombosed capillaries (TC). Left: HE, 10x. Right: HE, 20x.

and Scott 1993). The initial cause of the lesions can not be determined, but it is probable that some sort of trauma (*e.g.* mating, vestibulitis or vaginitis) have caused the first lesions. Subsequently, licking and biting of the lesions by this individual bear have caused further epithelial damage and persisting inflammatroy reaction with formation of granulated tissue. There was no signs of hyperplasia in the clitoral enlargement and therefore the female polar bear was not a pseudohermaphrodite (*i.e.* the enlargement was not due to endocrine disruption from androgen or antiestrogen exposure).

The uterine glands (crypts) were highly developed, indicating that this female had been in gestation earlier. Histological examination of the liver, kidneys, adrenals, spleen and lymph nodes showed only minor and insignificant changes (mainly mononuclear cell infiltrates) (Sonne et al., submitted-a,b).

## Concentrations of OCs and potential effects

### Organohalogens

Levels of ∑PCBs, ∑PBDEs, ∑DDTs, ∑HCHs and ∑CHLs in the adipose tissue of the 23-year-old female polar bear were compared with levels in 23 adult female polar bears (age: 5-25 years) from East Greenland (1999-2002) (Table 2). The concentrations of OHCs in this bear did not differ significantly from those in the reference group (all: $p>0.05$) except for ∑PCBs, which was slightly lower ($p=0.04$) (Table 2). ∑$MeSO_2$-PCB and 3-$MeSO_2$-4,4'-DDE concentrations were not included in these comparisons due to covariance, *i.e.*, ∑-$MeSO_2$-PCB to ∑-PCBs and 3-$MeSO_2$-4,4'-DDE to 4,4'-DDE ratios for the present Greenland bears were relatively constant values of 0.08 ± 0.03 and 0.06 ± 0.05, respectively (Sandala et al., 2004). Additional information on OC concentrations and information on sex, season differences and time trends in the East Greenland polar bear are available in Dietz et al., (2004) and Sandala et al., (2004).

Recent studies on polar bears from Svalbard have correlated high levels of OCs to disruption of retinol (vitamin A), thyroid, sex steroids and cortisol levels in blood (Skaare et al., 2001; Haave et al., 2003, Oskam et al., 2003, 2004) while other polar bear studies have indicated that high levels of OCs have a negative effect on IgG levels suggesting possible immunotoxic effects

on the IgG levels (Bernhoft et al., 2000, Lie et al., 2004, Lie et al., submitted). Several studies have shown that OHCs such as PBDEs, PCBs, DDTs, 3-$MeSO_2$-4,4'-DDE, $MeSO_2$-PCBs, HO-PCBs and other halogenated phenolic compounds including HO-PBDEs and 4-HO-heptachlorostyrene, have the potential of functioning as endocrine disruptors (Hakk and Letcher 2003, Legler and Brouwer 2003, Letcher et al., 2000, 2002, Sandau et al., 2000). With the exception of HO-PBDEs, tissue residue concentrations of congeners from all classes of these OHCs have been determined in polar bears. These investigations support the hypothesis that OCs have the potential to affect the immune and hormonal system, and can consequently elicit lower immune response.

**Table 2**
Basic statistics of the levels of contaminants in adipose tissue of the 23-year-old female polar bear with enlarged clitoris, with reference to 24 adult East Greenland female polar bears and one adult East Greenland male polar bear. PCBs, PBDEs, DDTs, HCHs and CHLs in ng/g l.w. Coplanare nPCBs, dioxins and furanes in liver: ng/kg (=pg/g) l.w. "s": sum; TEQ: Toxic Equivalents (pg/g l.w.)."." indicates missing TEF-values or missing values due to detection limit. *: measure not included in the 95% confidence interval of the observations.

| Variable | Female with enlarged clitoris | | Reference group | | |
|---|---|---|---|---|---|
| | | | Mean ± $t_{0.95}$ * SD | Min-max | N |
| Standard OCs: | Level | TEQ | | | |
| ΣPCBs | 2707.7 | . | 6984.4±9521.5 | 887.2-20346.9 | 24 |
| ΣPBDEs | 32.87 | . | 60.9±68.7 | 57.9-16.8 | 24 |
| ΣDDTs | 374.2 | . | 361.4±553.4 | 73.5-1112.5 | 24 |
| ΣHCHs | 128.3 | . | 235.3±444.7 | 14-817.6 | 24 |
| ΣCHLs | 898.8 | . | 1895.1±3072.8 | 353.6-8132.1 | 24 |
| Coplanar nPCBs: | | | | | |
| CB-37 | 1.76 | . | 3.6±9.1 | 0.58-7 | 4 |
| CB-77 | 4.26 | 0.00426 | 11.2±18.7 | 3.9-16.3 | 4 |
| CB-81 | 0.67 | 0.00067 | 4.1±16 | 0.62-11.48 | 4 |
| CB-126 | 25.27 | 2.527 | 62±76.2 | 26.61-79.71 | 4 |
| CB-169 | 73.31 | 0.07331 | 90.1±187.8 | 36.47-172.71 | 4 |
| CB-189 | 19.82 | 0.01982 | 99.5±310.4 | 6.68-236.23 | 4 |
| PCDDs: | | | | | |
| 1,2,3,7,8 PCDD | 1.16 | 0.58 | 1.96±2.7 | 0.78-2.64 | 4 |
| 1,2,3,4,7,8 HxCDD | 0.47 | 0.047 | 0.42±0.45 | 0.28-0.56 | 4 |
| 1,2,3,6,7,8 HxCDD | 1.88 | 0.188 | 2.3±2.8 | 1.25-3.20 | 4 |
| 1,2,3,4,6,7,9 HpCDD | 0.33 | . | . | . | 4 |
| 1,2,3,4,6,7,8 HpCDD | 0.70 | 0.007 | 0.54±0.88 | 0.35-0.74 | 4 |
| 1,2,3,4,6,7,8,9 OCDD | 4.14* | 0.00414 | 0.92±1.88 | 0.32-4.14 | 4 |
| PCDFs: | | | | | |
| 1,2,4,6,8 PCDF | 0.05 | . | 0. 1±0.19 | 0.05-0.17 | 4 |
| 1,2,4,7,8 PCDF | 0.33 | . | 0.32±0.67 | 0.14-0.61 | 4 |
| 2,3,4,7,8 PCDF | 0.65 | 0.325 | 1.46±2.09 | 0.65-2.13 | 4 |
| 1,2,3,4,7,8,9 HpCDF | 0.34 | 0.0034 | 0.38±0.68 | 0.23-0.53 | 4 |
| 1,2,3,4,6,7,8,9 OCDF | 0.82 | 0.00082 | 0.79±1.4 | 0.33-1.39 | 4 |
| Total TEQ | | 3.78 | | | |

The relatively low levels of analysed OHCs in the old female polar bear with enlarged clitoris are probably a result of gestation and lactation as it is known that up to 70% of the total body burden are transferred transplacentally from mother to foetus (Bernhoft et al., 1997, Polischuk et al., 2002). The levels of ∑PCBs (ng / g) in the present female polar bear have been compared to levels of effects published in de March et al., (1998) and AMAP, (2004) (Table 3A). The level of ∑PCBs in the aberrant female polar bear was at sample time 2708 ng / g l.w. which is not in the range thought to negatively influence reproduction or elicit immunotoxicological effects. ∑PCBs (3033 ng / g w.w) were well below the concentrations (Table 3A) reported to cause reproductive or immunotoxicological effects in harbour seal (*Phoca vitulina*) (16500 ng / g l.w.), ringed seals (77000 ng / g l.w.), otters (*Lutra lutra*) (4000 ng / g l.w.), mink (*Mustela vison*) (40000 ng / g l.w.) and Rhesus monkeys (*Macaca mulatta*) (21000 ng / g l.w.) (de March et al., 1998, AMAP, 2004).

#### Dioxins and dioxin-like compounds (TEQ values)

The premise of the TEQ concept applied to non-ortho and mono-ortho chlorine-substituted PCBs is to equate the "dioxin-like" induction capacity via the aryl hydrocarbon receptor (AhR)-mediated mechanism using "mammalian" toxic equivalency factors (TEFs), and thus expressing the ∑-coplanare PCBs concentrations after adjustment for the dioxin-like potency relative to 2,3,7,8-TCDD (van den Berg et al., 1998). AhR-mediated enzyme induction in mammals includes the CYP1A group (CYP1A1 and CYP1A2) and also recently identified CYP1B1 isozymes (Lin et al., 2003). Induced CYP1B1 and CYP1A1 enzymes can bioactivate *exogenous* compounds in the body (*e.g.* PCB congeners into HO-PCBs and other metabolites), and *endogenous* compounds in the body to toxic forms (*e.g.* CYP1B1-mediated metabolism of 17β-estradiol and estrone into carcinogenic 4-hydroxyestrogens), and / or can disrupt the homeostasis of estrogen hormones (van Duursen et al., 2003). *n*PCBs, dibenzofurans (PCDFs) and dibenzo-p-dioxins (PCDDs) in the present aberrant female polar bear were compared with three female polar bears (4-22 years) and one 6-year-old male polar bear from East Greenland (Table 2). One of the analysed PCDDs, 1,2,3,4,6,7,8,9 OCDD, in the aberrant bear differed from the values of 4 reference polar bears probably due to random biological variance (Table 2). The TEQ concentration comprised of *n*PCBs, PCDDs and PCDFs in the female polar bear with enlarged clitoris was 3.78 pg / g l.w. (Table 2) and is suspected to be lower than earlier in her life cycle (Bernhoft et al., 1997, Polischuk et al., 2002). In fact, based on time trends in the Arctic (*e.g.* AMAP, 2004, Dietz et al., 2004), the general OC exposure during her embryonic development was significant higher due to her heavily polluted mother, who is suspected to have carried organohalogen loads 2-3 timer higher (*Ibid.*). Compared to de March et al., (1998) and de AMAP, (2004) the total TEQ value (3.78 pg / g l.w.) for the present polar bears is 100-1000 times lower than thresholds known from seals and mink; and is generally comparable to terrestrial biota low on the food web (Table 3B).

**Table 3A**
Levels of ∑-PCBs (ng/g l.w.) and their toxic effects compared to the 23-year-old female polar bear with enlarged clitoris. Abbreviations: LOAEL: Lowest Observed Adverse Effect Level; NOAEL: No Adverse Effect Levels; NOEL: No Observed Effect Level; $EC_{50}$: Effect Concentration of 50% (data from de March et al., 1998).

| Level | Species | Effect (tissue) | Female with enlarged clitoris |
|---|---|---|---|
| 500 | Human | LOAEL short term memory | 3 033 |
| 1 000 | Human | NOAEL visual memory | 3 033 |
| 4 000 | Otter | Vitamin A reduction (liver) | 3 033 |
| 7 500 | Otter | NOEL reproduction | 3 033 |
| 9 000 | Mink | NOEL kit survival | 3 033 |
| 11 000 | Otter | Vitamin A reduction (liver) | 3 033 |
| 16 500 | Harbour seal | Immunosuppression, depressed vitamin A | 3 033 |
| 21 000 | Rhesus monkey | LOAEL immune effects | 3 033 |
| 25 000 | Harbour seal | Poor reproductive success | 3 033 |
| 40 000 | Mink | $EC_{50}$ litter size | 3 033 |
| 60 000 | Mink | $EC_{50}$ litter size | 3 033 |
| 77 000 | Ringed seal | Poor reproductive success | 3 033 |
| 80 000 | Mink | $EC_{50}$ kit survival | 3 033 |
| 120 000 | Mink | $EC_{50}$ kit survival | 3 033 |

**Table 3B**
Levels of known ∑-TEQ values (PCDDs/Fs, nPCBs; for otter also mono-ortho PCBs) and their toxic effects compared to the 23-year-old female polar bear with enlarged clitoris. Abbreviations: NOEL: No Observed Effect Level; LOAEL: Lowest Observed Adverse Effect Level; $EC_{50/90}$: Effect Concentration of 50/90% (data from de March et al., 1998, AMAP, 2004). All TEQ values in pg/g w.w. (wet weight) or pg/g l.w. (lipid weight).

| TEQ Level | Species | Effect (tissue analysed) | Female with enlarged clitoris (tissue) |
|---|---|---|---|
| 69 l.w. | Harbour seal | Immunosuppression | 3.78 pg/g l.w. (liver) |
| 84 w.w | Otter | NOEL for vitamin A reduction (liver) | 3.78 pg/g l.w. (liver) |
| 160 w.w. | Mink | EC50 litter size (muscle) | 3.78 pg/g l.w. (liver) |
| 200 w.w. | Mink | Kit survival (muscle) | 3.78 pg/g l.w. (liver) |
| 490 w.w. | Mink | Kit survival (liver) | 3.78 pg/g l.w. (liver) |
| 490 w.w. | Mink | LOAEL for mink kit survival (liver) | 3.78 pg/g l.w. (liver) |
| 520 l.w. | Marine mammals | Hepatic vitamin A stores (liver) | 3.78 pg/g l.w. (liver) |
| 520 l.w. | Marine mammals | Thyroid hormone concentrations (liver) | 3.78 pg/g l.w. (liver) |
| 520 l.w. | Marine mammals | Immunosuppression (liver) | 3.78 pg/g l.w. (liver) |
| 5000 l.w. | Otter | EC90 for vitamin A reduction (liver) | 3.78 pg/g l.w. (liver) |

### Skull

Untreated hyperadrenocorticism due to 21-hydroxylase deficiency can result in pseudohermaphroditism and decline in bone mineral density (osteoporosis) (Acland 1995, Feldman 1995, Mickelsen and Memon 1995, Valentino et al., 2000) and also, anthropogenic pollutants (*e.g.*, OCs) have been positively correlated to metabolic bone diseases (osteoporosis) in mammalian wildlife (Zakharov and Yablokov 1990, Bergman et al., 1992, Mortensen et al., 1992, De Guise et al., 1995, Schandorff 1997a-b, Lind et al., 2003). Therefore bone mineral density (BMD) in the female polar bear skull was examined to detect osteopenia or osteoporosis as a possible effect of endocrine disruption caused by high organohalogen exposure. The BMD of the aberrant female polar bear was close to the mean value found in a representative reference group of 16 adult female polar bears (age 5-23 years) from East Greenland

(Table 4), and we therefore conclude that this adult female bear did not deviate from the average of adult females. The negative correlation between organochlorines and BMD in subadults and adult males is reported elsewhere (Sonne et al., Accepted with revision).

**Table 4**
Basic statistics of skull bone mineral density (g hydroxyapatite) in the 23-year-old female polar bear with enlarged clitoris compared to at reference material of 16 adult East Greenland female polar bears (5-23 years) sampled during 1999-2002.

| Variable | Female with enlarged clitoris | Reference group | | |
|---|---|---|---|---|
| | | Mean ± $t_{0.95}$ * SD | Min-max | n |
| BMD (g/cm$^2$) | 1.78 | 1.88±0.45 | 1.49-2.15 | 16 |

## Regional comparisons

Pseudohermaphroditism is a rare phenomenon in Ursids as only 5 individuals have been reported for certain (Cattet 1988). The aberrant external genitalia of the East Greenland female polar bear was compared to the previously published *in vivo* examination of four bears from Svalbard (Wiig et al., 1998). In these four Svalbard polar bears, androgen producing tumours, 21-hydroxylase deficiency and/or organohalogens were proposed to be possible factors in the pathogenesis of the enlarged clitoris in these presumed pseudohermaphrodites. The observations of clitoral enlargement in the two adult female polar bears at Svalbard were similar to those of the East Greenland female (Table 5) and at least one of the Svalbard adults showed signs of earlier gestation and birth (milk in mammary glands) indicating that she had recently weaned and maybe lost her offspring. In addition signs of inflammation (pus and ulceration) was present (Ø. Wiig, pers.comm). This could link the enlarged clitoris in these adult bears to events associated with mating and chronic severe inflammatory responses in the perineal region (*i.e.* not pseudohermaphroditism).

Regarding the two Svalbard yearlings, these could not be evaluated for reproductive function. The Greenland finding of a trauma-related lesion that may have been caused by mating in March-April and subsequent inflammatory automotility reaction from licking does not fit with the two Svalbard cubs. They are unlikely to have been subjected to mating attempts or other trauma leading to lesion formation. The two cubs also differed morphologically from the Greenland adult in the caudal placement of the penis-like clitoris and the lateral opening of the urethra. Therefore the 'pseudohermaphroditic cubs' cannot be linked to the present female with clitoral enlargement and their anomalous organs might be explained by another phenomenon, *e.g.* 21-hydroxylase deficiency (21-CAH) congenital pseudohermaphrodite complex as proposed previously (Wiig et al., 1998). Furthermore, Freemartinism was not likely to cause the changes as the average litter size in spring is less than two and therefore a third male in the litter was unlikely (Ibid.).

**Table 5**
Comparison of changes in the external genitalia of free-living female polar bears from East Greenland (Scoresby Sound) and Svalbard.

| Information | Central East Greenland | Svalbard | |
|---|---|---|---|
| | Adult | Adults | Cubs |
| Number | One | Two | Two |
| Age | 23-yrs | 15-yrs | Yearling |
| Year | 1999 | 1990/97 | 1996 |
| Sex | F | F | F |
| Age | 23 years | 15 years | Yearlings |
| Vulva | Swollen | Swollen | Undeveloped |
| Female with cub(s) | No | No/weaned or lost cubs | No |
| Female in company with adult male | No | No/Yes | No |
| Vaginal opening | Normal | Normal | Normal |
| Clitoris length | ≥27 mm | ≥20 mm | ≥20 mm penis-like |
| Clitoris width | ≥22 mm | . | . |
| Clitoris height | ≥21 mm | . | . |
| Baculum | No (histology) | No (palpated) | Yes (palpated) |
| Location of clitoris/penis | Normal | Normal | Caudal to normal |
| Concealing | Normal pairs of labia | Normal | Vaginal opening by a single pair of labia |
| Urethral opening | Normal | Normal | 5mm laterally to the distal end of the penis-like clitoris |
| Y-chromosome (male) | No | ? | No |

## Conclusions

A 23-year-old female polar bear (*Ursus maritimus*) shot in East Greenland in 1999 had a significantly enlarged clitoris resembling in colour and form those of 'pseudohermaphroditic' adult female polar bears reported from Svalbard. The macrosopic and examination showed that its external and internal sexual organs, except the enlarged clitoris, were similar to those in a reference group of 23 normale female polar bears from East Greenland collected in 1999-2002. The bear was a female genotype, and macroscopic examination of her sexual organs indicated that she was reproductively functional. However, a histological examination of the clitoris revealed intense chronic ulcerative and perivascular *clitoriditis* showing that the enlargement was an inflammatory reaction, due to licking and biting, and not pseudohermaphroditism. The levels of major classes of OHCs were lower in this bear than in the reference group and wildlife and laboratory mammals suggesting that these compounds were not linked to the clitoral enlargement. Total TEQ values of PCDDs, PCDFs and *n*PCBs showed levels 100-1000 lower than known thresholds from wildlife and laboratory mammals. Neither could a difference in skull mineral density be found in the female compared to a reference material. Hence, the clitoral enlargement in the present adult female polar bear was due to an inflammatory response, why pseudohermaphroditism in adult female polar bears reported in the past could stem from misdiagnoses.

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**Wiig Ø.** Professor Øystein Wiig, University of Oslo, Norway (pers. comm.).

**Yager JA and Scott DW.** The skin and appendages (I). **In:** Jubb, K. V. F., Kennedy, P. C. and Palmer, N. (eds.): Pathology of Domestic Animals (4th edn.), Academic Press 1993: 531-738.

# Appendix

Entire publication list for Christian Sonne. Publications marked ”included” indicate that they are used in the present Ph.D. thesis.

## Published papers and manuscripts 2002-2004

1. **Sonne-Hansen C.**, R. Dietz, P. S. Leifsson, L. Hyldstrup and F. F. Riget (2002): Cadmium toxicity to ringed seals (Phoca hispida) - An epidemiological study of possible cadmium induced nephropathy and osteodystrophy in ringed seals (Phoca hispida) from Qaanaaq in Northwest Greenland. Sci Total Environ 295 (I-III): 167-181.

2. **Sonne C.**, F. F. Riget, R. Dietz, M. Kirkegaard, E. W. Born, R. J. Letcher and D. C. G. Muir (Submitted): Trends in fluctuating asymmetry in East Greenland polar bears (*Ursus maritimus*) from 1892 to 2002 in relation to organohalogen pollution. Sci Total Environ. ”Included”.

3. **Sonne, C.**, R. Dietz, F. F. Riget, E. W. Born, M. Kirkegaard, L. Hyldstrup, R. J. Letcher and D. C. G. Muir (Accepted with revision): Is bone mineral composition disrupted by organochlorines in East Greenland polar bears (*Ursus maritimus*)?. Environ Hlth Persp. ”Included”.

4. **Sonne, C.**, R. Dietz, E. W. Born, M. Kirkegaard, F. F. Riget, R. J. Letcher and D. C. G. Muir (In prep.): Periodontitis and tooth wear in East Greenland polar bears (*Ursus maritimus*) during 1892-2002. ”Included”

5. **Sonne C.**, R. Dietz, P. S. Leifsson, E. W. Born, M. Kirkegaard, F. F. Riget, R. J. Letcher, D. C. G. Muir and L. Hyldstrup (Submitted): Liver histology of free-ranging East Greenland polar bears (*Ursus maritimus*) in relation to organohalogen exposure. Toxicol Pathol. ”Included”.

6. **Sonne C.**, R. Dietz, P. S. Leifsson, F. F. Riget, E. W. Born, M. Kirkegaard, R. J. Letcher, D. C. G. Muir and L. Hyldstrup (Submitted): Renal lesions in East Greenland polar bears (*Ursus maritimus*) during 1999-2002. J Wildlife Dis. ”Included”.

7. **Sonne, C.**, P. S. Leifsson, R. Dietz, E. W. Born, R. J. Letcher, M. Kirkegaard, D. C. G. Muir, L. W. Andersen, F. F. Riget and L. Hyldstrup (In Press): Enlarged clitoris in wild polar bears (*Ursus maritimus*) can be misdiagnosed as pseudohermaphroditism. Sci Total Environ. ”Included”.

8. Dietz, R., F. F. Riget, **C. Sonne**, R. J. Letcher , E. W. Born and D. C. G. Muir (2004): Seasonal and temporal trends in Polychlorinated biphenyls and Organochlorine Pesticides in East Greenland polar bears (*Ursus maritimus*), 1990-2001. Sci Total Environ 331: 107-124. ”Included”.

9. Sandala, G. M., **C. Sonne**, R. Dietz, D. C. G. Muir, K. Valters, E. R. Bennett, E. W. Born and R. J. Letcher (2004): Hydroxylated and Methyl Sulfone PCB Metabolites in Adipose and Whole Blood of Polar Bear (*Ursus maritimus*) From East Greenland. Sci Total Environ 331: 125-141.

10. Heier, A., **C. Sonne**, A. Gröne, P. S. Leifsson, R. Dietz, E. W. Born and L. N. Bacciarini (Submitted): Liver RBP immunohistochemistry in free-ranging polar bears. An immunohistochemical study of retinol-binding protein (RBP) in livers of polar bears (*Ursus maritimus*) from East Greenland. Zoo and Wild Med.

11. Kirkegaard, M, **C. Sonne**, P.S. Leifsson, R. Dietz, E.W. Born, R. J. Letcher and D.C.G. Muir (Submitted): Histology of selected immunological organs in polar bear (*Ursus maritimus*) from East Greenland in relation to levels of organohalogenes. Sci Total Environ.

12. Kirkegaard, M., R. Dietz, **C. Sonne** and E. W. Born (In prep.): Age determination and use of dental structures to determine the reproductive history of female polar bears (*Ursus maritimus*).

13. Smithwick, M. S., A. de Silva, D. C. G. Muir, S. Mabury, K. Solomon, **C. Sonne**, R. Dietz and E. W. Born (In prep.): Perfluorinated acids in heptatic tissue from East Greenland polar bears (*Ursus maritimus*) 1999-2001.

14. Bossi, R., K. Vorkamp, F. F. Riget, R. Dietz, **C. Sonne** and P. Fauser (Submitted): Perfluorinated surfactants in fish, mammals and birds from Greenland and Faroe Islands. Results from a preliminary screening. Environ Pollut.

15. Muir, D. C. G., R. Dietz, F. F. Riget, **C. Sonne**, R. J. Letcher and E. W. Born (In prep.): Polybrominated diphenylethers in East Greenland polar bears (*Ursus maritimus*) 1990-2001.

16. Verreault, J., D. C. G. Muir, R. J. Norstrom, I. Stirling, A. T. Fisk, G. W. Gabrielsen, A. E. Derocher, T. Evans, R. Dietz, **C. Sonne**, G. M. Sandala, M. K. Taylor, J. Nagy and R. J. Letcher (Submitted): Chlorinated hydrocarbon contaminants and metabolites in polar bears (*Ursus maritimus)* from Svalbard, East Greenland, Alaska and the canadian arctic during 1999−2002. Sci Total Environ.

17. Merkel, F. R., A. Mosbech, **C. Sonne** and A. Flagstad (In prep.): Local movements, habitat use, and survival of common eiders wintering in southwest Greenland.

18. Mosbech, A., G. Gilchrist, F. R. Merkel and **C. Sonne** (In prep.): Comparing spring and autumn migration of Arctic common eider based on satellite telemetry.

19. Mosbech, A., **C. Sonne**, A. Flagstad, P. L. Fast, M. Fast, F. R. Merkel and H. G. Gilchrist (In prep.): Behavioral and surgical effects of intracoelomic implantation of satellite transmitters in common eider (*Somateria Molissima*) and king eider (*Somateria spectabiles*), North East Canada 2001-2003.

## Reviewed scientific reports 2000-2003

1. **Sonne-Hansen C.**, R. Dietz, P. S. Leifsson, L. Hyldstrup and F. F. Riget (2000): Cadmium toxicity to ringed seals (Phoca hispida). An epidemiological study of possible cadmium induced nephropathy and osteodystrophy in ringed seals (Phoca hispida) from Qaanaaq in Northwest

Greenland. National Environmental Research Institute, Denmark. 31pp – NERI Technical Report No. 307.

2. Dietz R., **C. Sonne-Hansen**, E. W. Born, H. T. Sandell and B. Sandell (2001): Aberrant polar bears in East Greenland. An interview investigation, 1999 (with english summary). National Environmental Research Institute, Technical Report no. 359, pp. 50, www.neri.dk.

3. Sandell, H.T. , B. Sandell, E.W. Born, R. Dietz and **C. Sonne-Hansen** (2001): Polar bears in East Greenland. An interview investigation of occurence and hunt, 1999 (with english summary). Technical Report no. 40, Pinngortitaleriffik, Greenland Institute of Natural Resources, 96 pp., www.ginr.gl.

4. **Sonne-Hansen, C.**, R. Dietz, F.F. Riget, E. W. Born, L. Hyldstrup and P. S. Leifsson (2003): Effects. Chapter 6. In: Riget, F. F., J. Christensen and P. Johansen (eds.). AMAP Greenland and the Faroe Islands 1997-2001. Ministry of Environment, Denmark.

## Oral workshop and conference presentations 1999-2004

1. **Sonne-Hansen C.**, R. Dietz, P. S. Leifsson, L. Hyldstrup and F. F. Riget (1999): Cadmium toxicity to ringed seals (Phoca hispida). An epidemiological study of possible cadmium induced nephropathy and osteodystrophy in ringed seals (Phoca hispida) from Qaanaaq in Northwest Greenland. Eurotox Conference 99, Oslo, Norway, June 27-30, 1999. Poster presentation.

2. Dietz, R., G. Gabrielsen, **C. Sonne-Hansen**, A. Derocher, E.W. Born and H. Wolker (2000): Impact of Contaminants on European Arctic Polar Bears "ICEBEAR". Rovaniemi, Finland, January 18-20, 2000. Poster presentation.

3. **Sonne-Hansen, C.**, R. Dietz, F. F. Riget, E. W. Born and M. Kierkegaard (2001): Contaminants in the Greenland Sea Polar Bear. The effect of persistent organic pollutants and new contaminants on internal endocrine organs and skulls in the East Greenland polar bear. NERI seminar 10.-12. Sept. 2001. Oral presentation.

4. Born, E.W., R. Dietz, **C. Sonne-Hansen**, H. Sandell and B. Sandell (2001): Aberrant polar bears in East Greenland, 14th Biennial Conference on the Biology of Marine Mammals, Vancouver, Canada november 23-2 december 2001. Poster presentation.

5. Mosbech, A., F. R. Merkel, A. Flagstad and **C. Sonne-Hansen** (2001): Satellite Tracking of King Eiders (Somateria spectabilis) in Western Greenland. Microwave Telemetry Conference 5.-7. Dec. in Columbia, MD, USA. Oral presentation.

6. **Sonne-Hansen, C.**, R. Dietz, F. F. Riget, E. W. Born and M. Kierkegaard (2002): Contaminants in the Greenland Sea Polar Bear. The effect of persistent organic pollutants and new contaminants on internal and endo-

crine organs and skulls in the East Greenland polar bear. The international AMAP conference Jan. 2002. Oral presentation.

7. Dietz, R, F. F. Riget, **C. Sonne-Hansen**, P. S. Leifsson, L. Hyldstrup, E. W. Born, D. C. G. Muir, R. J. Letcher, G. Asmund and M. Kirkegaard (2002): Pollution of East Greenland polar bear around the millennium change. Second AMAP International Symposium on Environmental Pollution of the Arctic, Rovaniemi, Finland, 1 – 4 Oct. 2002. Oral presentation.

8. **Sonne-Hansen, C.**, R. Dietz, F. F. Riget, E. W. Born and M. Kierkegaard (2003): Contaminants in the Greenland Sea Polar Bear. The effect of persistent organic pollutants and new contami-nants on internal and endocrine organs and skulls in the East Greenland polar bear. Ph.D. workshop, Department of Anatomy & Physiology, Copenhagen, Denmark, 7 Feb. 2003. Oral presentation.

9. Sandala, G. M., **C. Sonne-Hansen**, R. Dietz, D. C. G. Muir, E. R. Bennett and R. J. Letcher (2003): Methyl sulfone and hydroxylated PCB metabolites in adipose versus whole blood of polar bear (*Ursus maritimus*) from Scoresby Sound, Greenland. Canadian Arctic Contaminants Assessment Symposium, Ottawa, Canada, March 4-7, 2003. Poster presentation.

10. Kirkegaard, **C. Sonne**, P. S. Leifsson, R. Dietz, E. W. Born, R. J. Letcher and D. C. G. Muir (2004): Histology of selected immunological organs in East Greenland polar bears in relation to levels of organohalogens. 18th Annual Conference, European Cetacean Society, March 28.-31., Kolmården, Sweden, 2004. Poster presentation.

11. Merkel, F.R., Mosbech, A. and **Sonne-Hansen, C**. (2004): Habitat selection and foraging strategies of common Eiders wintering in south-west Greenland. Waterbirds around the World, A global review of the conservation, management and research of the world's major flyways, Edinburgh, UK, 3-8 April, 2004. Poster presentation.

12. Mosbech, A., Merkel, F., **Sonne-Hansen, C.** and Gilchrist, G. (2004): Use of satellite telemetry to locate key habitats for King Eiders in Western Greenland. Waterbirds around the World, A global review of the conservation, management and research of the world's major flyways, Edinburgh, UK, 3-8 April, 2004. Oral presentation.

# National Environmental Research Institute

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Organohalogen concentrations and a gross and histologic assessment of multiple organ systems in East Greenland polar bears

To investigate the relation between biological parameters, not earlier investigated in the polar bear, and organohalogen pollution in East Greenland polar bears, we initiated a sampling of adipose tissue, internal organs and skulls from more than 100 free-ranging polar bears killed by local subsistence hunters from Central East Greenland (69°00'N to 74°00'N) during 1999-2002. The present thesis exposes the first and most important results from this large multidisciplinary study of this material, and evaluates the possible connection between the relatively high levels of organohalogens in the adipose tissue and pathological changes in skulls and internal organs. Our results suggested a decrease in adipose tissue concentrations of organohalogens in East Greenland polar bears from 1990 to 1999-2001. Two of the biological effect parameters (FA and enlarged clitoris) did not indicate a link to the relatively high levels of organohalogens. But, there was indications of strong relationships between various organohalogen compounds and skull mineral density indicating disruption of the bone mineral composition. The histopathological changes found in liver- and kidney tissue were a result of ageing, infectious agents, season and meaby chronic exposure to organohalogens. These result fill out an existing knowledge gap in potential effects of environmental, organic contaminants on fluctuating asymmetry, bone mineral density and functional anatomy (histology) in the polar bear. In addition, the results may have a large social importance for Inuits as well.

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