

## ARE ORGANOHALOGEN CONTAMINANTS A COFACTOR IN THE DEVELOPMENT OF RENAL LESIONS IN EAST GREENLAND POLAR BEARS (*URSUS MARITIMUS*)?

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(Received 19 August 2005; Accepted 28 October 2005)

**Abstract**—Tissues of polar bears (*Ursus maritimus*) from East Greenland contain the highest concentrations of organohalogen contaminants (OHCs) among subpopulations of any mammalian species in the Arctic. Negative associations also have been found between OHC concentrations and bone mineral density and liver histology parameters for this subpopulation of polar bears. The present study examined the OHC concentrations and adverse effects on renal tissue for 75 polar bears collected during 1999 to 2002. Specific lesions were diffuse glomerular capillary wall thickening, mesangial glomerular deposits, tubular epithelial cell hyperplasia, hyalinization of the tubular basement membrane, tubular dilatation, atrophy and necrosis, tubular medullary hyalin casts, interstitial fibrosis, and mononuclear cell infiltration. With the exception of mononuclear cell infiltrations, all these parameters were correlated with age, whereas none was associated with the sex of the animals. In an age-controlled statistical analysis of covariance, increases in glomerular mesangial deposits and interstitial fibrosis were significantly ( $p < 0.05$ ) correlated with polychlorinated diphenyl ether ( $\Sigma$ PBDE) concentrations in subadults. In adult males, statistically significant ( $p < 0.05$ ) positive correlations were found for tubular epithelial cell hyperplasia and dieldrin concentration; diffuse glomerular capillary wall thickening and chlordane ( $\Sigma$ CHL) concentrations, and tubular medullary hyalin casts and  $\Sigma$ CHL,  $\Sigma$ PBDE, polychlorinated biphenyl, and hexachlorocyclohexane concentrations. The lesions were consistent with those reported previously in highly OHC-contaminated Baltic seal populations and exposed laboratory animals. The renal lesions were a result of aging. However, based on the above statistical findings as well as the nature of the findings, we suggest that long-term exposure to OHCs may be a cofactor in renal lesion occurrence, although other cofactors, such as exposure to heavy metals and recurrent infections from microorganisms, cannot be ruled out. This is new and important knowledge in the assessment of health status among wildlife populations and humans relying on food resources that are contaminated with OHCs.

**Keywords**—Histopathology East Greenland Organohalogen compounds Polar bears Renal lesions

### INTRODUCTION

Polar bears (*Ursus maritimus*) from East Greenland, Svalbard (Norway), and the Kara Sea carry higher loads of anthropogenic organohalogen contaminants (OHCs; e.g., polychlorinated biphenyls [PCBs], DDT, and polybrominated diphenylethers [PBDEs]) compared with polar bears elsewhere in the Arctic, because these three areas receive air- and sea-borne pollutants from the same lower latitudinal sources (see, e.g., [1,2]; <http://www.amap.no/>). In polar bears, OHCs are transferred transplacentally from mother to fetus and via lactation to the juvenile and subadults (see, e.g., [2,3]). Therefore, adverse health effects in all life stages are suspected because of the agonistic and/or antagonistic properties with respect to endogenous hormones in various organs and tissues (see, e.g., [1,4]).

At Svalbard, recent studies of PCBs/organochlorine pesticides in polar bears have indicated a negative association to plasma testosterone (males), progesterone (females), cortisol (both sexes), retinol (both sexes), and thyroxine hormone (both sexes) [5–9]. Additionally, high levels of organochlorines were

associated with low levels of IgG in the Svalbard bears, suggesting possible immunotoxic effects [10–12].

Similar investigations of bone mineral density (BMD) in East Greenland polar bear skulls ( $n = 139$ ) sampled during 1892 to 2002 showed a strong negative correlation between BMD and DDT, PCB, dieldrin, and chlordanes (CHLs), as well as a lower BMD in the period from 1966 to 2002 (polluted period) when compared to the period from 1892 to 1936 (non-polluted period) [13]. We suggested that this may indicate increased risk for osteoporosis and bone fractures in relation to OHC exposure [13]. The OHCs are believed to negatively affect reproduction and survival of free-ranging seals in the Baltic Sea (see, e.g., [1,14–16]). Furthermore, studies of free-ranging gray seals (*Halichoerus grypus*) and ringed seals (*Phoca hispida*) from the Baltic Sea [17] have shown an association between organochlorines and renal lesions. In dose-response and case-control experiments with OHCs, toxic effects on renal tissue have been found in rats [18–20], bream fish (*Abramis brama*), and asp fish (*Aspius aspius*) [21].

Finally, OHCs are suspected to be cofactors in liver and splenic/lymph node changes in the East Greenland subpopulation of polar bears [22,23]. To investigate whether the high levels of OHCs negatively affect other internal target organs,

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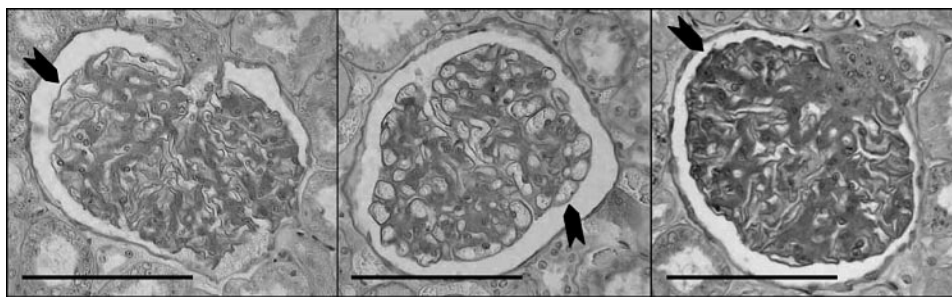


Fig. 1. Thickening of the renal glomerular capillary wall in East Greenland polar bears sampled 1999 to 2002. (left) Absent. (middle) Mild. (right) Moderate. Arrowheads indicate thickening of the glomerular capillary wall. Periodic acid–Schiff staining, magnification  $\times 40$ , bar = 50  $\mu\text{m}$ .

renal tissue from 75 East Greenland polar bears was examined histologically and subsequently compared to adipose tissue concentrations of OHCs. The present investigation is important for better understanding of adverse effects of OHCs on polar bear health and, by extension, the potential impact on human populations in the Arctic who rely on contaminated food resources.

### MATERIALS AND METHODS

#### *Sampling and preparation*

All polar bear samples were taken by local subsistence hunters in the Scoresby Sound area in central East Greenland (ca. 69°N to ca. 74°N) during 1999 to 2002. A randomly chosen, single renal lobe was collected from 75 individuals and fixed in a phosphate-buffered formaldehyde/alcohol solution (3.5% formaldehyde, 86% ethanol, and 10.5% H<sub>2</sub>O) [24,25]. In addition, subcutaneous adipose tissue was sampled from 62 individuals and stored in separate polyethylene plastic bags until arrival at the National Environmental Research Institute laboratory in Roskilde, Denmark, where they were transferred into rinsed acetone (Supra solv. 1.00012; Merck, KGaA, Darmstadt, Germany) and *n*-hexane (Uni-solv 1.04369; Merck) glass containers covered with identically rinsed aluminum foil between the sample and the plastic lid. All samples were taken less than 12 h postmortem and were preserved frozen during the hunt and later kept at  $-20^{\circ}\text{C}$  before preparation and examination for histology at the Department of Veterinary Pathobiology (Copenhagen, Denmark) and for PCB/organochlorine pesticide and PBDE analyses at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, ON, Canada; Letcher Laboratories) and National Water Research Institute (Burlington, ON, Canada), respectively.

#### *Age determination*

The age determination was carried out by counting the cementum growth layer groups of the lower I<sub>3</sub> tooth after de-

calcification, thin sectioning (thickness, 14  $\mu\text{m}$ ), and staining (toluidine blue) using the method described by Hensel and Sorensen [26]. When necessary, the individuals were categorized as subadults, adult males, and adult females according to the following criteria: males six years or older as adult males, females five years or older as adult females, and all remaining individuals as subadults [27]. When evaluating sex difference in the prevalence of renal lesions, bears were categorized as old when they were 15 years or older based on the criteria described by Derocher and Stirling [28].

#### *Histology*

Renal tissue was trimmed, processed conventionally, embedded in paraffin, sectioned (thickness,  $\sim 4$   $\mu\text{m}$ ), and stained with hematoxylin-and-eosin for routine diagnostics. Periodic acid–Schiff (PAS) and periodic acid–silver methenamine were used to demonstrate glomerular (capillary and mesangial) and tubular changes, Van Gieson and Masson trichrome to detect fibrous tissue (collagen) in the glomeruli (glomerulofibrosis) and in the interstitium (interstitial fibrosis), and Smorl and Perls Prussian blue reaction to detect lipofuscin and hemosiderin pigments [24]. Glomerular, tubular, and interstitial lesions were evaluated semiquantitatively by examining the renal tissue in 10 randomly selected low- to high-power fields (magnification,  $\times 5$ – $40$ ). Based on these observations, the lesions were graded into one of three groups (0, absent; 1, mild; 2, moderate). The criteria for multifocal mild and moderate glomerular diffuse capillary wall thickening is shown in Figure 1, and the criteria for multifocal mesangial glomerular deposits, often accompanied by collagenous fibrosis, are shown in Figure 2. In cases of tubular hyalin casts, hyalinization of basement membrane, dilatation, atrophy, and necrosis, these were graded as mild or moderate when present focally or multifocally, respectively (Fig. 3). The criteria for dividing tubular epithelial cell hyperplasia, interstitial fibrosis, and mononuclear cell infiltrations into groups of mild and moderate lesions

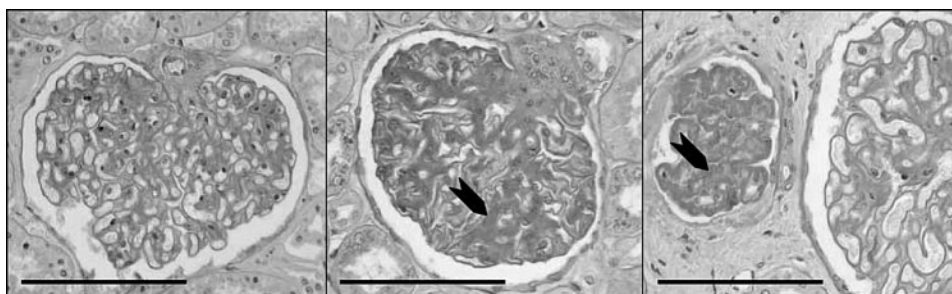


Fig. 2. Renal glomerular periodic acid–Schiff–positive mesangial deposits in East Greenland polar bears sampled 1999 to 2002. (left) Absent. (middle) Mild. (right) Moderate. Arrowheads indicate deposits. Magnification  $\times 40$ , bar = 50  $\mu\text{m}$ .

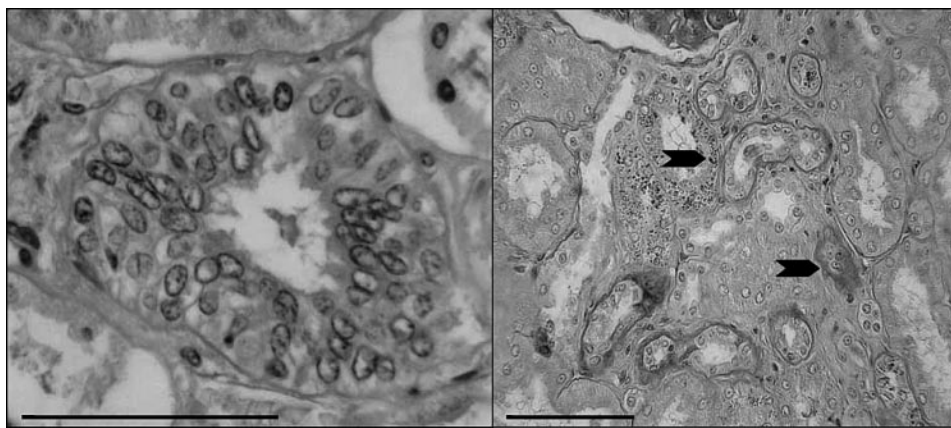


Fig. 3. Renal tubular lesions in East Greenland polar bears sampled 1999 to 2002. **(left)** Hyperplasia of distal convoluted tubular epithelial cells. **(right)** Hyalinization of the tubular basement membrane, tubular atrophy, and necrosis as well as interstitial fibrosis (see Fig. 4). Arrowheads indicate hyalinization and tubular pigments. Periodic acid–Schiff staining, magnification  $\times 40$ , bar = 50  $\mu\text{m}$ .

was the presence of focal and multifocal lesions, respectively (Fig. 4). Tubular yellow-brown pigment was found in all individuals, but the nature and significance of these could not be determined (Fig. 3). In a few individuals, swelling of Bowman's capsule and segmental capsular adhesion between the glomerular capillary wall and Bowman's capsule, as well as glomerular atrophy, were found but not investigated quantitatively.

#### OHC analyses

Subcutaneous samples of polar bear adipose tissue ( $n = 62$ ) were analyzed for PCBs, DDTs, CHLs, dieldrin, hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB) according to the method described by Dietz et al. [29] and Sandala et al. [30] at the Great Lakes Institute for Environmental Research. The external standard quantification approach used for PCBs and organochlorines in the subcutaneous adipose tissues was based on peak area of the gas chromatography  $\mu$ -electron capture detection response, which has been described in detail by Dietz et al. [29]. Briefly,  $\Sigma\text{PCB}$  is the sum of the concentrations of the 51 individual or coeluting congeners (if detected): 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, and 206. The  $\Sigma\text{DDT}$  is the sum of 4,4'-DDT, 4,4'-dichlorodiphenyldichloroethane, and 4,4'-dichlorodiphenyldichloroethylene. The

$\Sigma\text{HCH}$  is the sum of the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -hexachlorocyclohexane. The  $\Sigma\text{CHL}$  is the sum of oxy-CHL, *trans*-CHL, *cis*-CHL, *trans*-nonachlor, *cis*-nonachlor, and heptachlor epoxide. The OHC chemical fractions were subsequently sent to the National Water Research Institute for determination of PBDE flame retardants. The  $\Sigma\text{PBDE}$  ( $n = 62$ ) were determined by electron-capture negative-ion (low-resolution) mass spectrometer using an external standard as described previously by Luross et al. [31]. Briefly,  $\Sigma\text{PBDE}$  is the sum of the concentrations of the 35 individual or coeluting congeners (if detected): 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, and 190.

#### Statistics

The statistical analyses were performed with the SAS statistical software package (SAS 9.1 and Enterprise Guide 3.0; SAS Institute, Cary, NC, USA), and the significance level was set to  $p = 0.05$ . The OHC data were log-transformed (base-e) before the analyses to meet the assumption of normality and homogeneity of the variance.

For each specific lesion, a one-way ANOVA was performed to test for differences in mean age between individuals with and without lesions. Furthermore, we tested for a relationship between sex and renal lesions within subadults, adults, and old individuals using a  $\chi^2$ -test.

A one-way ANOVA was performed to test for differences

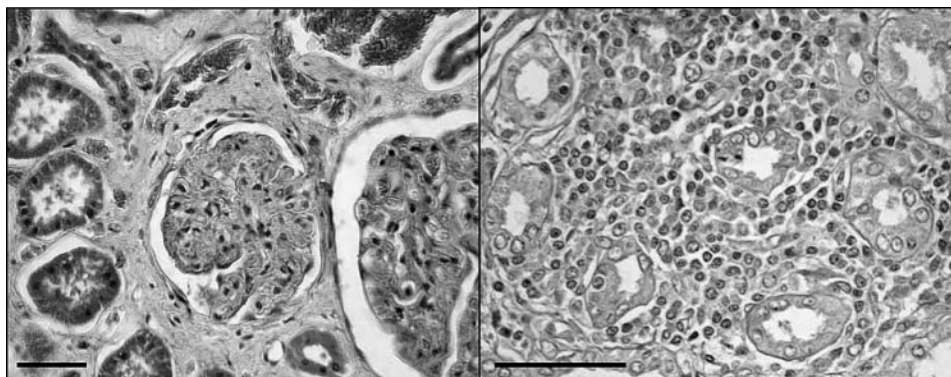


Fig. 4. Renal interstitial lesions in East Greenland polar bears sampled 1999 to 2002. **(left)** Interstitial and glomerular fibrosis (collagen) with total glomerular obliteration (sclerosis). **(right)** Intense cortical mononuclear cell infiltration. Periodic acid–Schiff staining, magnification  $\times 40$ , bar = 50  $\mu\text{m}$ .

Table 1. Prevalence of renal lesions in relation to age and sex in 75 East Greenland polar bears sampled during 1999 to 2002<sup>a</sup>

Renal lesion	Absent (% [n])	Mild (% [n])	Moderate (% [n])	Age ( <i>p</i> [F])	Sex ( <i>p</i> [F])
Glomerular capillary wall thickening	78 (59)	19 (14)	3 (2)	** (11.4)	NS
Glomerular mesangial deposits	26 (20)	39 (29)	35 (26)	** (7.9)	NS
Tubular epithelial cell hyperplasia	79 (59)	9 (7)	12 (9)	*** (35.2)	NS
Tubular hyalinization/atrophy/dilatation/necrosis	64 (48)	13 (10)	23 (17)	*** (41.4)	NS
Tubular medullary hyalin casts <sup>b</sup>	85 (62)	14 (10)	1 (1)	* (5.8)	NS
Interstitial fibrosis	70 (53)	15 (11)	15 (11)	*** (43.5)	NS
Mononuclear cell infiltrations	49 (37)	35 (26)	16 (12)	NS	NS

<sup>a</sup> Statistical significance is presented as follows: \* individuals with renal lesions significantly older (mean age) than individuals without renal lesions at  $p \leq 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ . NS = no significant relationship between renal lesion and age nor sex at  $p > 0.05$ .

<sup>b</sup> Two individuals were excluded from the evaluation because of suboptimal medullary fixation.

in mean concentrations of each group of OHCs (PCBs, DDTs, CHLs, dieldrin, HCHs, HCB, and PBDEs) between subadults, adult females, and adult males. These results were then evaluated by a Tukey's post-hoc test. To test the relationship between concentrations of OHC and age, a linear-regression model was employed for subadults, adult females, and adult males.

Relationships between the concentrations of each group of OHCs (PCBs, DDTs, CHLs, dieldrin, HCHs, HCB, and PBDEs) and each renal lesion (absent and mild/moderate) were tested by an analysis of covariance (ANCOVA) for subadults, adult females, and adult males to control for the effect of age. For the ANCOVA, OHC concentrations were dependent variables, age was a covariable, and renal lesions were a class variable (with their first-order interaction links: Age  $\times$  renal lesion). The statistical analyses were employed separately on subadults, adult females, and adult males, because the relationship between OHC concentrations and age (significant age  $\times$  age/sex group interactions in the full model), as well as most of the OHC concentrations, differed between these three age/sex groups. After a successive reduction of nonsignificant interactions, judged from the type III sum of squares ( $p > 0.05$ ), the significance of each of the remaining factors was evaluated from the final model (least-square means).

## RESULTS AND DISCUSSION

A total of 75 free-ranging East Greenland polar bears (40 subadults, 12 adult females, 13 adult males, 6 old females, and 4 old males) collected from 1999 to 2002 were included in the present study. The macroscopic anatomy of these 75 polar bears appeared to be multilobulated (see, e.g., [25,32]).

### Glomerular lesions

Mild and moderate diffuse thickening of the glomerular capillary wall was found in 22% of the individuals (Fig. 1 and Table 1) and was similar to membranous glomerulonephritis reported in wildlife, domestic animals, and humans [17,33–36]. In addition, mild and moderate PAS-positive mesangial glomerular deposits, often accompanied by collagenous fibrosis, were found in 74% of the animals. In the case of interstitial fibrosis, dense Masson trichrome- and PAS-positive, total fibrous obliteration of the glomeruli (sclerosis) was present (Figs. 2 and 4 and Table 1) [33,35,36]. In a few cases, swelling of Bowman's capsule and segmental capsular adhesion between the glomerular capillary wall and Bowman's capsule were found, leaving luminal, PAS-positive hyalin deposits in Bowman's space. Both glomerular lesions exhibited positive age relationships (all:  $p < 0.01$ ), whereas the sex of the animal was not influential (all: nonsignificant) (Table 1).

### Tubular lesions

The most prominent change was hyperplasia of epithelial cells in the distal convoluted tubules and collecting ducts [17,34,36], which was found in 21% of the bears (Fig. 3 and Table 1). The cells were large and pale, with a polygonal and monomorphic appearance, and protruded into the luminal area, either as a monolayer or as solid islands within the lumen. The second most prominent feature was mild and moderate PAS-positive hyalinization of the tubular basement membrane accompanied by tubular atrophy, dilation, necrosis, and interstitial fibrosis [17,34,36] and was found in 35% of the animals (Figs. 3 and 4 and Table 1). In moderate cases, these lesions were accompanied by interstitial fibrosis and total glomerular obliteration [17,34,36] (Figs. 2 and 4 and Table 1). Furthermore, tubular intracellular yellow-brown pigments, localized basally and apically, were found in the entire nephron of all individuals (Fig. 3 and Table 1). The pigments were tested and found to be negative for both lipofuscin and hemosiderin (Smorl and Perls') [24] and, therefore, could be bile pigments, hemoglobin, melanin, or by-products from the metabolism of plant material digested during summer. However, the nature and significance of these findings were not investigated further. Tubular cylindrical hyaline casts (protein) were found in the medulla of 15% of the individuals, indicating tubular protein loss (Table 1). As for glomerular lesions, all three tubular lesions exhibited positive age relationships (all:  $p \leq 0.05$ ), whereas the sex of the animal was not influential (all: nonsignificant) (Table 1).

### Interstitial lesions

Mild and moderate interstitial fibrosis [17,25,34,36] was found in 30% of the bears, and in the most severe cases, total PAS-positive glomerular obliteration (fibrosis) and tubular lesions were found as described above (Figs. 2 to 4 and Table 1). Mononuclear cell infiltration in the cortex, medulla, and papilla was recorded in 51% of the bears and subsequently categorized as chronic focal, nonsuppurative interstitial nephritis [17,25,34,36] (Fig. 4 and Table 1). In a few cases, intense cell infiltrations were accompanied by tubular dilatation, atrophy, and necrosis, but rarely fibrosis or tubular hyalin casts. Mononuclear cell infiltrations were not related to age, whereas interstitial fibrosis was highly related to age ( $p < 0.001$ ). However, neither of these two lesions were influenced by the sex of the animals (Table 1).

### Levels of OHCs

Levels of  $\Sigma$ PCB,  $\Sigma$ CHL,  $\Sigma$ DDT, dieldrin,  $\Sigma$ HCH, HCB, and  $\Sigma$ PBDE of 62 of the examined polar bears are presented

Table 2. Organohalogen compound (OHC) concentrations (mean  $\pm$  standard deviation, ng/g lipid wt) in subcutaneous adipose tissue of 62 East Greenland polar bears investigated for renal lesions during 1999 to 2001<sup>a</sup>

OHC	Subadults (n = 33)	Adult females (n = 17)	Adult males (n = 12)
$\Sigma$ PCB	5,635 $\pm$ 2,024	5,099 $\pm$ 2,255*#	7,928 $\pm$ 3,811
$\Sigma$ DDT	450 $\pm$ 156	336 $\pm$ 219*##	445 $\pm$ 320
$\Sigma$ CHL	1,326 $\pm$ 654	1,328 $\pm$ 603	924 $\pm$ 539**
Dieldrin	199 $\pm$ 75	169 $\pm$ 70	146 $\pm$ 91*##,####
$\Sigma$ HCH	175 $\pm$ 58	174 $\pm$ 173	247 $\pm$ 186
HCB	96 $\pm$ 78	74 $\pm$ 84	41 $\pm$ 30*##
$\Sigma$ PBDE	55 $\pm$ 34	67 $\pm$ 42	47 $\pm$ 20

<sup>a</sup> Statistical significance is presented as follows: \*# = Significantly lower when compared to adult males at  $p \leq 0.05$ ; \*## = significantly lower when compared to subadults at  $p \leq 0.05$ ; #### = significantly negative relationship with age at  $p \leq 0.05$  ( $r^2 = 0.4$ ); \*\* = significantly negative relationship with age at  $p < 0.01$  ( $r^2 = 0.58$ ). HCB = hexachlorobenzene; HCH = hexachlorocyclohexane; PBDE = polybrominated diphenyl ether; PCB = polychlorinated biphenyl.

in Table 2. The  $\Sigma$ CHL,  $\Sigma$ HCH, and  $\Sigma$ PBDE did not differ significantly between age/sex groups, whereas  $\Sigma$ PCB,  $\Sigma$ DDT, dieldrin, and HCB differed between the three age/sex groups (all:  $p \leq 0.05$ ); however, no consistent trend was found (Table 2). For adult males, a significant negative relationship was found between age and  $\Sigma$ CHL ( $p < 0.01$ ,  $r^2 = 0.58$ ) and between age and dieldrin ( $p = 0.03$ ,  $r^2 = 0.4$ ) (Table 2). Further information about age and sex variation of OHCs in the present East Greenland polar bears can be found in Dietz et al. [29] and Sandala et al. [30].

#### Histopathology and OHCs

Statistical analyses were employed separately for subadults, adult females, and adult males, because OHC concentrations and the relationship to age (significant age  $\times$  age/sex group interactions) differed between these three age/sex groups (Table 2). We tested if the concentrations of each OHC group differed between individuals with and without lesions (absent and mild/moderate, respectively) when controlling for age dependency (ANCOVA). For subadults, the presence of glomerular mesangial PAS-positive deposits increased significantly with concentrations of  $\Sigma$ PBDE ( $p \leq 0.05$ ), and the same was the case for interstitial fibrosis ( $p < 0.01$ ) (Table 3). In adult males, the presence of hyperplasia of epithelial tubular cells increased significantly with concentrations of dieldrin ( $p$

$\leq 0.05$ ), diffuse glomerular capillary wall thickening with  $\Sigma$ CHL ( $p \leq 0.05$ ), and tubular medullary hyalin casts with  $\Sigma$ CHL ( $p \leq 0.05$ ),  $\Sigma$ PBDE ( $p \leq 0.05$ ),  $\Sigma$ PCB ( $p < 0.01$ ), and  $\Sigma$ HCH ( $p < 0.001$ ), respectively (Table 3).

#### Glomerular and tubular lesions

Diffuse thickening of the glomerular capillary wall, caused by immune deposits on the epithelial side of the glomerular capillary basement membrane, has been described in domestic animals, wildlife, and humans (see, e.g., [17,33–37]). This type of lesion has been associated with drug abuse and exposure to toxic substances, such as OHCs and heavy metals, as well as with chronic recurrent infections (see, e.g., [17,33–37]). The fact that this lesion was highly correlated to the age of the present bears agrees well with knowledge from other species (see, e.g., [17,33–37]), but based on the associations between  $\Sigma$ CHL and thickening of the glomerular capillary wall in adult males, OHCs may be a cofactor as well. Meanwhile, heavy metals and chronic inflammation from microorganisms (bacteria, virus, and parasites) also cannot be ruled out (see, e.g., [17,33–37]).

Age-related glomerular changes, such as PAS-positive mesangial deposits and fibrosis (sclerosis), has been found in the domestic dog (*Canis familiaris*), humans, and wildlife [17,33–37]. In the present study, this change was exacerbated in cases of interstitial fibrosis, which is an expected age-related change (see, e.g., [17,33–37]). However, prolonged exposure of the potent estrogen stilbestrol to rodents, pigs, and domestic dogs has been shown to initiate increased amounts of interstitial fibrous tissue, with atrophy of glomeruli and tubuli, in these species [38–41]. Because PCBs and DDTs are known xenoestrogens (see, e.g., [1]), it cannot be excluded that these substances are cofactors in the development of renal lesions in the present East Greenland polar bears and of recurrent infections. McCormack et al. [19] found atrophied glomeruli in rats and dogs exposed to polybrominated biphenyls (PBBs; Firemaster BP6®; Michigan Chemical, St. Louis, MI, USA) as well as an increase in the aryl hydrocarbon hydroxylase and catalytic activity in the renal tissue. Although PBBs and PBDEs do not exhibit similar toxic properties, it is noteworthy that we found a relationship between PBDEs and mesangial deposits, interstitial fibrosis, and medullary hyalin casts in the bears, although the concentration of brominated flame retardants differed significantly between the two studies.

Tubular epithelial cell hyperplasia has been associated with

Table 3. Significant results from analyses of relationships between renal lesions and organohalogen compounds (OHCs) in subadults of both sexes and adult male East Greenland polar bears from 1999 to 2001<sup>a</sup>

Age/sex group	Renal lesion	OHC	$p$ (n, F)
Adult males	Tubular medullary hyalin casts	$\Sigma$ CHL	*(12, 5.9)
	Tubular medullary hyalin casts	$\Sigma$ PBDE	*(12, 6.8)
	Tubular medullary hyalin casts	$\Sigma$ PCB	** (12, 23.0)
	Tubular medullary hyalin casts	$\Sigma$ HCH	*** (12, 40.0)
	Tubular epithelial cell hyperplasia	Dieldrin	*(12, 9.5)
	Glomerular capillary wall thickening	$\Sigma$ CHL	*(12, 5.8)
Subadults	Glomerular mesangial deposits	$\Sigma$ PBDE	*(33, 5.9)
	Interstitial fibrosis	$\Sigma$ PBDE	** (33, 10.9)

<sup>a</sup> Statistical significance is presented as follows: \* Significantly higher OHC level (age-corrected least square means) in individuals with mild/moderate lesions than in individuals without lesions at  $p \leq 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ . CHL = chlordanes; HCB = hexachlorobenzene; HCH = hexachlorocyclohexane; PBDE = polybrominated diphenyl ether; PCB = polychlorinated biphenyl.

regeneration of renal parenchyma and chronic renal failure [33–36]. However, the morphology of tubular epithelial cell hyperplasia in the polar bears was similar to that of PCB-induced (Aroclor 1242) hyperplasia of papillary epithelia after long-term exposure in rats [18]. Also, long-term exposure of Baltic seals to xenoestrogens (e.g., PCBs and DDTs) have been shown to initiate similar squamous metaplasia of the urogenital tract epithelia (distal tubules and collecting ducts), as have the potent estrogen stilbestrol in rodents, pigs, and domestic dogs [17,38–41]. The metaplasia in these studies was found in the very distal parts of the nephrons, and the finding of similar hyperplasia in the East Greenland polar bears, and their relationship to dieldrin concentrations in adult males, may indicate an impact of xenoestrogen-mediated activity (i.e., PCBs and DDTs) in renal tissue of these bears. However, based on the knowledge from chronic renal failure studies, human transplantations and domestic animals, other vascular dysfunctions, recurrent infections, or autoimmunity also cannot be ruled out [17,33–36]. However, age is the main factor in the present study, although dieldrin and recurrent infections cannot be excluded as playing a cofactor role in the etiology of the lesions.

#### *Interstitial lesions*

We cannot conclude whether the interstitial fibrosis in the present investigation was caused by age, recurrent infections (micropathogens), or OHCs (e.g., the PBDE concentration relationship in subadults). The interstitial nephritis (mononuclear cell infiltrations) found in the present study was similar to that found in ringed seals from North West Greenland [25] and Baltic seals [17] and probably resulted from chronic, recurrent infections (microorganisms; see, e.g., [25,32–36]).

#### *OHC exposure as a cofactor?*

The fact that positive relationships were found between  $\Sigma$ PCB,  $\Sigma$ CHL, dieldrin,  $\Sigma$ HCH,  $\Sigma$ PBDE, and specific lesions in subadults and adult males supports the possibility that OHC may be a cofactor in the development of the lesions, although heavy metals and recurrent infections should be taken into account (we have no data to substantiate this yet). Recently, we found strong indications of BMD being linked to the levels of  $\Sigma$ PCB and  $\Sigma$ CHL exposure in the same subadult individuals, and by  $\Sigma$ DDT and dieldrin in the same adult polar bear male individuals as in the present study [13]. Indications of a positive relationship between  $\Sigma$ HCH and HCB concentrations and the presence of liver histopathology were found in the same adult male individuals as in the present study [22]. It is therefore possible that a cofactor linkage may exist between OHC exposure and renal lesions.

The histopathological changes found in glomeruli, tubuli, and interstitium were similar to those reported in Baltic gray seals and ringed seals heavily polluted by OHCs (PCBs, DDTs, and others) and heavy metals between 1977 and 1996 [17]. Those authors suggested that the lesions were a result of age and, based on a low-exposed reference material, also were a result of chronic exposure to OHCs. Unfortunately, we could not provide renal tissue from a control group of nonexposed, free-ranging polar bears that had lived under the same environmental conditions as the present East Greenland polar bears, because such a group does not exist.

Finally, based on the nature of the lesions and the significant relationships to adipose concentrations of OHCs, we suggest that age was the major etiological factor, but that chronic sub-

lethal exposure to OHCs probably functions as a cofactor (based on the statistical associations and nature of the lesions). We could not evaluate whether the relatively high levels of mercury (and cadmium) accumulated in the renal tissue of the present East Greenland polar bear subpopulation could influence our histological results (mercury, 2.87–32.0  $\mu$ g/g wet wt; cadmium, 2.16–28.9  $\mu$ g/g wet wt) [42]. However, in the case of mercury, the levels are in the range of adverse toxic effect levels for terrestrial mammals as provided by Thompson [43]. Chronic recurrent infections may play a role as well.

In future investigations, the observed lesions may be useful as biomarkers of OHC exposure in wildlife. This is all new information, which may be useful in studies of wildlife and human environmental toxicology. Whether the renal lesions, and the possible demineralization of the skeletal system and liver histopathology, have an impact on the health status of each individual polar bear (a cohort or the entire subpopulation) is impossible to evaluate. However, the most susceptible individuals may suffer from a lethal impact.

### CONCLUSION

The 75 East Greenland polar bears examined in the present study exhibited seven different renal lesions. Six of the seven lesions were related to age, and we therefore believe that age was the major factor in the development of the lesions. However, the lesions were all similar to those reported in OHC-contaminated Baltic seals as well as exposed laboratory animals. In addition, significant relationships between renal lesions and OHCs in subadults and adult males were found when controlled for age. We therefore suggest that exposure to OHCs may be a cofactor in the development of renal lesions in East Greenland polar bears, but that heavy metals (cadmium and mercury) and recurrent infections (microorganisms) cannot be ruled out. Some of the lesions found may be useful in the evaluation and understanding of wildlife and humans relying on OHC-contaminated food resources.

*Acknowledgement*—Danish Cooperation for Environment in the Arctic, the Commission for Scientific Research in Greenland, and Canada Research Chairs Program (to R.J. Letcher) are recognized for their financial support of the present study. Hanne Tuborg Sandell, Birger Sandell, Jonas Brønlund, and local hunters are thanked for the polar bear sampling in East Greenland.

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