

1 **Are Liver and Renal Lesions in East Greenland Polar Bears (*Ursus maritimus*)**
2 **Associated with High Mercury Levels?**

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24 **Abstract**

25 **Background:** In the Arctic, polar bears (*Ursus maritimus*) bio-accumulate mercury as they prey on
26 polluted ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*). Studies have shown
27 that polar bears from East Greenland are among the most mercury polluted species in the Arctic. It
28 is unknown whether these levels are toxic to liver and kidney tissue. **Methods:** We investigated the
29 histopathological impact from anthropogenic long-range transported mercury on East Greenland
30 polar bear liver (n=59) and kidney (n=57) tissues. **Results:** Liver mercury levels ranged from 1.1-
31 35.6 µg/g wet weight and renal levels ranged from 1-50 µg/g wet weight, of which 2 liver values
32 and 9 kidney values were above known toxic threshold level of 30 µg/g wet weight in terrestrial
33 mammals. Evaluated from age-correcting ANCOVA analyses, liver mercury levels were
34 significantly higher in individuals with visible Ito cells (p<0.02) and a similar trend was found for
35 lipid granulomas (p=0.07). Liver mercury levels were significantly lower in individuals with portal
36 bile duct proliferation/fibrosis (p=0.007) and a similar trend was found for proximal convoluted
37 tubular hyalinisation in renal tissue (p=0.07). **Conclusions:** Based on these relationships and the
38 nature of the chronic inflammation we conclude that the lesions were likely a result of recurrent
39 infections and ageing but that long-term exposure to mercury could not be excluded as a co-factor.
40 The information is important as it is likely that tropospheric mercury depletion events will continue
41 to increase the concentrations of this toxic heavy metal in the Sub Arctic and Arctic marine food
42 webs.

43

44 **Key words:** East Greenland, Histopathology, Kidney, Liver, Mercury, Polar bear, Renal, *Ursus*
45 *maritimus*.

46 **Background**

47 Approximately 200-300 tons mercury (Hg) is yearly transported to the Arctic from lower latitudes
48 via the atmosphere and large scale ocean currents [1, 2], hence the Arctic acts as a sink for global
49 emitted mercury due to spring mercury depletion events [3-6]. Mercury is toxic and the organic
50 methyl-mercury (MeHg) form mediates central nervous toxicity pre- and postnatally mainly via the
51 posterior cortex [7, 8]. Mercury levels are high in the Greenland and Faroe populations and a
52 special focus has been to address health effects in these regions as such effects are proposed to have
53 a significant impact on the socio-economy [9].

54
55 The inorganic form of mercury is toxic to liver and kidney tissues due to the co-enzyme inhibition
56 via high affinity to various microsome and mitochondria SH-group enzymes [8]. Studies of Atlantic
57 bottlenose dolphins (*Tursiops truncatus*) [10] and Arctic beluga whales (*Delphinapterus leucas*)
58 [11] have linked mercury exposure to liver histopathological changes. Also, renal glomerular and
59 tubular lesions have been associated with mercury toxicity in controlled laboratory studies [12-14],
60 in humans [15, 16], in wildlife [11, 17] and in domestic mammals [18, 19].

61
62 In the Arctic, polar bears (*Ursus maritimus*) bio-accumulate mercury as their preying on polluted
63 ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*) which results in a high
64 mercury uptake [1, 2, 20]. Studies have shown that East Greenland polar bears are among the most
65 mercury polluted species in the Arctic [1, 2]. In target organs like liver and kidney, inorganic
66 mercury concentrations of 2.13–13.4 and 2.87–32.0 µg/g w.w. (wet weight) have been reported [21,
67 22]. These levels exceed threshold levels for lethal mercury toxicity [1, 2]. Although mercury is a
68 naturally occurring element in the Arctic, Dietz et al. [23] showed that up to 94% of the mercury in
69 East Greenland polar bears is of anthropogenic origin. We therefore examined liver and kidney
70 histology and mercury concentrations in East Greenland polars sampled during 1999 to 2002 to
71 determine whether high mercury concentrations may cause pathological changes at the histological
72 level in target organs of large Arctic top predators.

73 **Methods**

74

75 *Sampling*

76 Liver (n=59) and kidney (n=57) samples were taken by local subsistence hunters in the Scoresby
77 Sound area in central East Greenland (69°00'N to 74°00'N) during 1999-2002. A randomly chosen
78 single renal lobe and a tissue sample from the periphery of a right liver lobe was taken for
79 histological examination and fixed in a phosphate buffered formaldehyde/alcohol solution (3.5%
80 formaldehyde, 86% ETOH and 10.5% H₂O) to avoid freeze damage. In addition, sub samples for
81 mercury analyses were stored in separate Polyethylene plastic bags until arrival at the laboratory in
82 Roskilde. All samples were taken <12 h *post mortem*.

83

84 *The sample*

85 The sample consisted of 32 subadults (10 females and 22 males), 15 adult females (5 ≥15 years) and
86 12 adult males (4 ≥15 years). Of these, 32 were caught during summer (1 June to 30 September)
87 and 27 during winter (1 October to 31 May). The general East Greenland polar bear liver and renal
88 histology, including micrographs, is described by Sonne et al. [24, 25].

89

90 *Age estimation*

91 The age determination was carried out by counting the cementum Growth Layer Groups (GLGs) of
92 the lower I₃ tooth after decalcification, thin sectioning (14µm) and staining (toluidine blue) using
93 the method described by Dietz et al. [26]. Adult males were categorised as ≥ 6 years, adult females
94 as ≥ 5 years, old as ≥ 15 years and the remaining as subadults [27, 28]. Furthermore, seasonal
95 differences were investigated as summer: 1 June to 30 September vs. winter: 1 October to 31 May
96 [27].

97

98 *Liver histology*

99 The liver tissue was trimmed, processed conventionally, embedded in paraffin, sectioned at about 4
100 μm and stained with Haematoxylin (Al-Haematein)-Eosin (HE) and periodic acid-Schiff (PAS) for
101 routine diagnostics, Van Gieson and Masson Trichrome to detect fibrous tissue (collagen), Best's
102 carmine to demonstrate glycogen storage, Sudan III to detect lipid (frozen tissue) and Perls'
103 Prussian blue reaction and Smorl for detecting haemosiderin and lipofuscin pigments, respectively
104 [29, 30].

105
106 Six histological features were evaluated: 1. Portal mononuclear cell infiltrations (absent, unifocally,
107 multifocally or diffuse). 2. Random mononuclear cell infiltrations (average no. in 5 fields at 10x
108 magnification). 3. Lipid granulomas (average no. in 5 fields at 10x magnification). 4. Hepatocytic
109 steatosis (intracellular lipid; absent, foamy, multifocally macrovesicular or diffuse
110 macrovesicular). 5. Visible Ito cells (average no. in 5 fields at 20x magnification). 6. Mild
111 multifocally bile duct proliferation accompanied by portal fibrosis (absent or present). Each
112 histological change was grouped semi-quantitatively as: 1. Portal mononuclear cell infiltrations:
113 absent=0; mild=unifocally; moderate=multifocally and severe=diffuse. 2. Random cell infiltrations:
114 absent=0; mild=Index]0;1]; moderate=Index]1;3] and severe=Index]3; ∞]. 3. Lipid granulomas:
115 absent=0; mild=Index]0;1[; moderate=Index [1;2[and severe=Index [2;5]. 4. Hepatocytic steatosis:
116 mild=foamy; moderate=multifocally macrovesicular and severe=diffuse macrovesicular. 5. Ito
117 cells: absent=0; mild=Index]0;10]; moderate=Index]10;50] and severe=Index]50;200]. 6. Bile
118 duct proliferation; see above.

119
120 *Renal histology*

121 Kidney tissue was trimmed, processed conventionally, embedded in paraffin, sectioned at about 4
122 μm and Periodic acid-Schiff (PAS) and periodic acid silver methenamine (PAS-M) were used to
123 demonstrate glomerular (capillary and mesangial) and proximal convoluted tubular changes; Van
124 Gieson and Masson Trichrome to detect fibrous tissue (collagen) in the glomeruli
125 (glomerulofibrosis) and in the interstitium (interstitial fibrosis) [30]. Seven histological features

126 were evaluated: 1. Glomerular capillary wall thickening (10 randomly selected 5-40x fields). 2.
127 Glomerular mesangial deposits (10 randomly selected 5-40x fields). 3. Tubular epithelial cell
128 hyperplasia (10 randomly selected 5-40x fields). 4. Proximal convoluted tubular
129 hyalinization/atrophy/dilations/necrosis (10 randomly selected 5-40x fields). 5. Tubular medullar
130 hyaline casts (10 randomly selected 5-40x fields). 6. Interstitial fibrosis (10 randomly selected 5-
131 40x fields). 7. Mononuclear cell infiltrations (10 randomly selected 5-40x fields). Each histological
132 change was grouped semi-quantitatively as: 1. Glomerular capillary wall thickening: absent, mild
133 and moderate. 2. Glomerular mesangial deposits: absent, mild and moderate. 3. Tubular epithelial
134 cell hyperplasia: mild: focally; moderate: multi focally. 4. Proximal convoluted tubular
135 hyalinization/atrophy/dilations/necrosis: mild: focally; moderate: multi focally. 5. Tubular medullar
136 hyaline casts: mild: focally; moderate: multi focally. 6. Interstitial fibrosis: mild: focally; moderate:
137 multi focally. 7. Mononuclear cell infiltrations: mild: focally; moderate: multi focally.

138

139 *Analyses of mercury*

140 Liver ($n=59$) and kidney ($n=57$) samples were analysed for mercury levels ($\mu\text{g/g w.w.}$) according to
141 Dietz et al. [23]. The principle was atomic absorption spectrometry (AAS; hydride generation and
142 the flow injection analyses) have previously been described by Asmund et al. [31]. The detection
143 limit was 0.005 mg/kg of dry weight. Analytical quality was ensured by repeated analyses and by
144 frequent analysis of various certified reference materials [TORT-2 (lobster hepatopancreas),
145 DORM-2, and Dolt-3] supplied by the National Research Council of Canada (Marine Analytical
146 Chemistry Standards Program).

147

148 *Statistical analyses*

149 The statistical analyses were performed with the SAS statistical software package (SAS V8 and
150 enterprise guide V1) and the level of significance was set to $p \leq 0.05$, while significance at
151 $0.05 < p \leq 0.10$ was considered a trend. The mercury data were log-transformed (base e) prior to the
152 analyses in order to meet the assumption of normality and homogeneity of the variance.

153

154 For each specific histologic change, a One-way ANOVA was performed to test for differences in
155 mean age between individuals with and without that specific histologic change (Table 1). In case of
156 hepatocytic steatosis, foamy cytoplasm was tested against macro vesicular lipid accumulation.

157 Furthermore, we tested whether there was a relationship between sex and season ([summer: 1 June
158 to 30 September]; [winter: 1 October to 31 May]), respectively, and histologic lesions using a χ^2 -
159 test. In case of age dependency, a χ^2 -test was performed within subadults, adults and old,
160 respectively, to test for sex differences.

161

162 The difference between mercury concentration and sex was tested in an age-normalizing analysis of
163 covariance (ANCOVA) with mercury concentration as the dependent variable, age as covariable
164 and sex as class variable, including their 1st order interaction links (age×sex). After a successive
165 reduction of non-significant interactions, judged from the type-III sum of squares ($p \leq 0.05$), the
166 significance of each of the remaining factors was evaluated from the final model Least Square
167 Mean (LSMean). Then, a One-way ANOVA was performed to test for differences in mercury mean
168 concentration between subadults, adult females and adult males. The results were finally evaluated
169 from Tukey's *post hoc* test (Table 2). In order to test the relationship between concentrations of
170 mercury and age, a linear regression model was employed for subadults, adult females and adult
171 males, respectively (Table 2).

172

173 Finally, the relationship between mercury concentration and each histologic change (absent vs.
174 present) was tested by an age correcting analysis of covariance (Table 3). This was conducted with
175 mercury concentration as dependent variable, age as covariable and histologic change (absent vs.
176 present) as class variable, including their 1st order interaction links (age×histologic change). The
177 statistical analyses were employed on all pooled individuals and additionally on subadults and
178 adults, respectively. After a successive reduction of non-significant interactions, judged from the
179 type-III sum of squares ($p \leq 0.05$), the significance of each of the remaining factors was evaluated

180 from the final model Least Square Mean (LSMean).

181 **Results**

182

183 *Liver lesions*

184 Multifocal mononuclear cell infiltrations (lymphocytes, and macrophages and neutrophils) were
185 found in 11%, portal mononuclear cell infiltrations in 15% and lipid granulomas in 63% of the
186 bears, respectively (Table 1). All animals showed hepatocytic foamy cytoplasm (microvesicular
187 steatosis), while 64% presented lipid accumulating Ito cells and 76% exhibited macrovesicular
188 steatosis (lipid vacuoles) in the periacinar zone 2 and 3. Mild bile duct proliferation accompanied by
189 portal fibrosis was found in 8% of the animals. Only Ito cell lipid accumulation and bile duct
190 proliferation were associated with age (both: $p<0.01$) while none of the lesions were related to sex
191 or season (all: $p>0.05$).

192

193 *Renal lesions*

194 Glomerular diffuse capillary wall thickening (similar to membranous glomerulonephritis due to
195 immune deposits on the epithelial side of the glomerular capillary basement membrane) and PAS
196 positive glomerular mesangial deposits/fibrosis was found in 28% and 78% of the animals,
197 respectively (Table 1). Interstitial fibrosis, including dense Masson Trichrome and PAS-positive
198 total fibrous obliteration of the glomerular sclerosis, was found in 25% of the individuals. All
199 glomerular lesions exhibited positive age relationships ($p<0.05$). Hyperplasia of distal convoluted
200 tubule and collecting duct epithelial cells was found in 25% of the bears. PAS-positive hyalinization
201 of the proximal convoluted tubular basement membrane accompanied by proximal convoluted
202 tubular dilatation, atrophy, necrosis and interstitial fibrosis was found in 39% of the animals. In
203 moderate cases, these lesions were accompanied by interstitial fibrosis and total glomerular
204 obliteration. Tubular cylindrical hyaline casts (protein) were found in the medulla of 16% of the
205 individuals, indicating protein loss. All tubular lesions exhibited positive age relationships ($p<0.05$).
206 The prevalence of mild and moderate interstitial fibrosis was 25%, while mononuclear cell
207 infiltration in cortex, medulla and papilla was recorded in 61% of the polar bears. Mononuclear cell

208 infiltrations were not related to age ($p>0.05$). None of the renal lesions were related to sex or season
209 (summer vs. winter) (all: $p>0.05$).

210

211 *Mercury concentrations*

212 Liver mercury levels ranged from 1.1-35.6 $\mu\text{g/g}$ w.w. and renal levels ranged from 1-50 $\mu\text{g/g}$ w.w.,
213 of which 2 liver values and 9 kidney values were above the known threshold toxic levels of 30 $\mu\text{g/g}$
214 w.w. in terrestrial mammals (Table 2, Figure 1, 2). Mercury in subadults was significantly lower
215 when compared to both males and females ($p<0.05$), while kidney mercury levels in adult males
216 were significantly higher when compared to adult females ($p<0.05$). In general, the deviation was
217 most pronounced in adult females. When analysing mercury vs. age relationship within subadults,
218 adult females and adult males, respectively, the liver and kidney mercury increased significantly
219 with age in subadults (both: $p<0.002$), while that was not the case for adults. The analyses of
220 covariance normalising for age revealed no difference in mercury concentrations when comparing
221 all females and all males for both kidney ($p>0.05$) and liver ($p>0.05$) tissue, although it seemed that
222 kidney concentrations decreased in old females and increased in old males. Neither was there a
223 seasonal difference (summer vs. winter) in liver and kidney mercury concentrations (both: $p>0.05$).

224

225 *Mercury concentrations and histological lesions*

226 Based on these results, the relationship between histopathology (present vs. absent) and mercury
227 was analysed on the entire pooled material and in subadults and adults, separately, within the age-
228 correcting analyses of covariance. The analyses showed that mercury liver levels were significantly
229 higher in individuals exhibiting visible Ito cells when compared to those not exhibiting visible Ito
230 cells ($p<0.02$) (Table 3, Figure 3). A similar trend was found for liver lipid granulomas ($p>0.05$)
231 (Table 3, Figure 3). In case of portal fibrosis, mercury levels were significantly lower in individuals
232 exhibiting portal fibrosis when compared to those not exhibiting portal fibrosis ($p=0.007$) and the
233 same trend was found in case of hyalinisation of renal tubular basement membranes ($p=0.07$) (Table
234 3, Figure 3).

235 **Discussion**

236

237 *Liver histology*

238 We found several different histopathological changes that could not be ascribed to specific
239 etiological factors. The mononuclear cell infiltrates were non-specific inflammatory reactions
240 towards micro organisms and/or injury of local blood vessels [32, 33]. The bile duct proliferation
241 and fibrosis were probably non-specific tissue reactions towards infections and thereby a result of
242 ageing [32, 33]. Prunescu et al. [34] speculated whether interstitial fibrosis was due to pre-
243 hibernation physiological adaptations in brown bear (*U. arctos*). However, such a seasonal pattern
244 could not be detected in the East Greenland polar bears. The number of lipid granulomas, in turn,
245 was probably originated from Ito cell rupture. The lipid accumulation in polar bear Ito cells has
246 previously been described and it constitutes the major accumulation and storage sites for lipophilic
247 vitamin A [24, 35-37]. Young bears only gradually start eating vitamin A rich prey until they are
248 weaned at app. 2 years of age, which could explain the age difference in Ito cell numbers [36, 37].
249 Specifically, the zonary (periacinary) hepatocytic steatosis could be ascribed to high lipid ingestion
250 and starvation while we cannot rule out other co-factors such as abnormal hepatocytic function and
251 decreased syntheses of apo proteins [32, 33].

252

253 However, the considerable mercury concentrations accumulated in the liver tissue of the polar bears
254 were in the range of adverse toxic effect levels for terrestrial mammals [1, 2]. Specifically,
255 hepatocytic steatosis was similar to mercury intoxication (hypoxia) in general [32, 33]. The signs of
256 chronic inflammation, bile duct proliferation, portal fibrosis and hepatocytic steatosis were similar
257 to those reported in mercury exposed bottlenose dolphins [10] and Arctic beluga whales [11].
258 Studies on laboratory rodents support these findings [38]. Furthermore, Dietz et al. [23] showed that
259 as much as 94% of the mercury in East Greenland polar bear hair sampled during 1892-2001 was
260 likely of anthropogenic origin. Such an increase demands certain tolerance from polar bears to
261 avoid sub cellular organ damage via *e.g.* up regulated methallothionein synthesis and selenid

262 complex binding [1, 2, 10, 40]. Previous studies of East Greenland polar bears have shown that the
263 liver mercury:selenium molar ratio exceeded 1:1 for a few individuals which indicate that some of
264 the total mercury is on a ion toxic form [22]. The fact that portal bile duct proliferation and fibrosis
265 was associated with decreasing mercury concentrations may be due to decreased liver
266 metabolism/function (uptake) or a simultaneously hepatocytic subcellular injury or death [39, 41,
267 42]. Furthermore, the positive association between mercury concentration and number of Ito cells
268 and lipid granulomas could indicate a direct impact from mercury on the prevalence of liver
269 inflammation [24]. In conclusion, the liver lesions were a result of recurrent infections and age
270 while mercury could not be ruled out as potential co-factor.

271

272 *Renal histology*

273 Diffuse thickening of the glomerular capillary wall, glomerular deposits/sclerosis and tubular
274 changes are all well described in domestic animals [18, 19], wildlife [11, 17, 40] and humans [15,
275 16]. These lesions are all associated with age and recurrent infections while exposure to toxic
276 substances is a known co-factor [15, 16]. In the polar bears, glomerular sclerosis was worsen in
277 cases of interstitial fibrosis, which is an expected and age-related change in various mammals, while
278 the observed tubular epithelial cell hyperplasia has been associated with regeneration of renal
279 parenchyma and chronic renal failure [15, 16]. The interstitial nephritis (mononuclear cell
280 infiltrations) was similar to those found in seals from North West Greenland and the Baltic [17, 40]
281 which we ascribe to chronic recurrent infections [15, 16, 18, 19].

282

283 However, the histopathological lesions found in glomeruli (immune-complex glomerulonephritis),
284 tubules and interstitium resemble those in Baltic grey seal and ringed seal heavily polluted with
285 heavy metals between 1977-1996 [17]. Similar associations are also reported from controlled
286 laboratory studies [12-16], in humans [15-16], in domestic mammals [18, 19] and other Arctic
287 wildlife species [11]. Proximal convoluted tubular basement membrane hyalinisation was
288 associated with decreasing mercury levels. That could be due to a decreased metabolism/kidney

289 function or because of mercury induced liver injury. In that case, the leakage of mercury (included
290 in polypeptide complexes) into the plasma and hence increasing the burden of Hg filtered through
291 the glomeruli, which in turn would generate tubular hyalinization increased excretion or mercury
292 induced liver injury [39, 41, 42]. According to Dietz et al. [22], the East Greenland polar bear renal
293 tissue mercury:selenium molar ratio exceeded 1:1 for several individuals, which indicate that a large
294 amount of the total mercury is on the ion toxic form, suggesting a toxic potential. However, the
295 conclusion is that the chronic nature of the lesions was a result of age and recurrent infections while
296 we cannot exclude mercury as a co-factor.

297

298 *Considerations*

299 Whether the present lesions have an impact on health status at the individual level is impossible to
300 evaluate. However, it cannot be excluded that individuals that are more susceptible to mercury
301 toxicity may be affected as 16% and 3% of the animals exceeded renal and liver threshold levels,
302 respectively, for toxic effects of the quoted metal in mammals [2]. Furthermore, Sonne et al. [24,
303 25] showed that anthropogenic organochlorines and brominated flame retardants were possible co-
304 factors in the development of renal and liver lesions in East Greenland polar bears. The present
305 study shows associations between organ lesions and mercury levels which indicate mercury as a co-
306 factor although recurrent infections and age clearly were main factors. Anyhow, the mercury levels
307 adds another risk factor to polar bear health as the pollution cocktail increase and thereby the
308 possibility of additive effects. Furthermore, the polar bear health mercury problem may be most
309 pronounced in the Western Arctic, as the highest concentrations exists there [1, 2, 23] due to
310 increasing emissions from Eurasia.

311 **Conclusions**

312 We found liver and kidney mercury levels in East Greenland polar bears in the range of toxic
313 effects. The signs and nature of chronic inflammation and statistical relationships points towards
314 age and recurrent infections as main factor while mercury could not be ruled out as a possible co-
315 factor. These are new and important results in the monitoring and assessment of the potential toxic
316 impact from the increasing mercury concentrations in Arctic wildlife and humans relying on
317 polluted marine species.

318 **Competing interests**

319 Conflicts of financial or non-financial interest were not reported.

320 **Authors' contributions**

321 CS drafted the manuscript. CS and RDI outlined the study design and RDI conducted the age
322 determinations with MKI. PSL participated in the histopathological examinations. GA conducted
323 the mercury analyses, EWB participated in the study design. All authors read and approved the final
324 manuscript.

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475 **TABLES**

476

477 **Table 1.** Prevalence (%[n]) of liver and kidney histopathology in East Greenland polar bears sampled during
 478 1999-2002.

| Organ | Absent | Mild | Moderate | Severe | Age <i>p</i> (F) |
|---|---------------|-------------|-----------------|---------------|-------------------------|
| <i>Liver (n=59)</i> | | | | | |
| Multifocally mononuclear cell infiltrations | 89 (53) | 11 (6) | - | - | n.s. |
| Portal mononuclear cell infiltrations | 85 (50) | 8 (5) | 7 (4) | - | n.s. |
| Lipid granulomas | 37 (22) | 36 (21) | 22 (13) | 5 (3) | n.s. |
| Hepatocytic steatosis | - | 24 (14) | 24 (14) | 52 (31) | n.s. |
| Ito cell lipid accumulation | 36 (21) | 14 (8) | 19 (11) | 31 (19) | ** (7.8) |
| Bile duct proliferation with fibrosis | 92 (54) | 8 (5) | - | - | *** (15.6) |
| <i>Kidney (n=57)</i> | | | | | |
| Glomerular diffuse capillary wall thickening | 72 (41) | 25 (14) | 3 (2) | - | *** (8) |
| Glomerular mesangial deposits/sclerosis | 22 (13) | 39 (22) | 39 (22) | - | * (5.6) |
| Interstitial fibrosis | 75 (42) | 12 (7) | 13 (8) | - | *** (49.8) |
| Tubular epithelial cell hyperplasia | 75 (42) | 12 (7) | 13 (8) | - | ** (6.7) |
| Tubular hyalinization/atrophy/dilatation/necrosis | 61 (35) | 12 (7) | 27 (15) | - | *** (30.8) |
| Tubular medullar hyaline casts | 84 (48) | 13 (8) | 3 (1) | - | * (6.6) |
| Mononuclear cell infiltrations | 39 (22) | 42 (24) | 19 (11) | - | n.s. |

479 Lesions are divided into groups of absent, mild, moderate and severe. n.s.: individuals with lesions not significantly

480 older (mean age) than individuals without lesions at $p > 0.05$. *: individuals with lesions significantly older (mean age)

481 than individuals without lesions at $p \leq 0.05$, **: at $p \leq 0.01$ and ***: at $p \leq 0.001$.

482 **Table 2.** Mercury concentrations [mean \pm SD, $\mu\text{g/g}$ w.w.] in liver and kidney tissue from East Greenland
483 polar bears collected during 1999-2002. Number of observations is given in parentheses.

| Organ | Subadults | Adult females | Adult males |
|--------|-------------------------|-------------------------|----------------------------|
| Liver | 6.27 \pm 2.83 (32)*# | 15.78 \pm 8.6 (15)* | 14.99 \pm 8.96 (12)* |
| Kidney | 7.18 \pm 3.32 (31)### | 18.04 \pm 10.97 (15)* | 29.42 \pm 14.09 (11)*,** |

484 *: Significantly higher when compared to subadults at $p \leq 0.05$. **: Significantly higher when compared to adult females
485 at $p < 0.05$. #: significantly positive relationship to age at $p \leq 0.002$ ($R^2 = 0.27$). ###: significantly positive relationship to
486 age at $p \leq 0.001$ ($R^2 = 0.45$).

487 **Table 3.** Significant results from the analyses of relationships between histology (presence vs. absence) and
488 mercury levels in East Greenland polar bears collected during 1999-2002.

| Age Group | Organ | Histologic change | <i>p</i> (<i>n</i>, <i>F</i>, <i>R</i>²) |
|------------------|--------------|---|--|
| All | Liver | Ito cell* | 0.01 (50; 6.4; 0.53) |
| Adults | Liver | Lipid granulomas* | 0.07 (22; 3.6; 0.38) |
| All | Liver | Portal bile duct proliferation and fibrosis** | 0.007 (50; 8; 0.47) |
| Subadults | Kidney | Tubular hyalinisation, atrophy, dilatation and necrosis** | 0.07 (31; 3.4; 0.51) |

489 The calculations of differences between individuals with and without lesions are based on ANCOVA Least Square
490 Mean regression analyses normalising for age. *: individuals with lesions higher in mercury levels than individuals
491 without lesions. **: individuals with lesions lower in mercury levels than individuals without lesions.

492 **FIGURE LEGENDS**

493

494 **Figure 1.** Liver levels of mercury ($\mu\text{g/g w.w.}$) in 34 male (left) and 25 female (right) East
495 Greenland polar bears. Known threshold level for mercury toxicity in wildlife is given based on
496 Dietz et al., (1998a) and AMAP (2005).

497

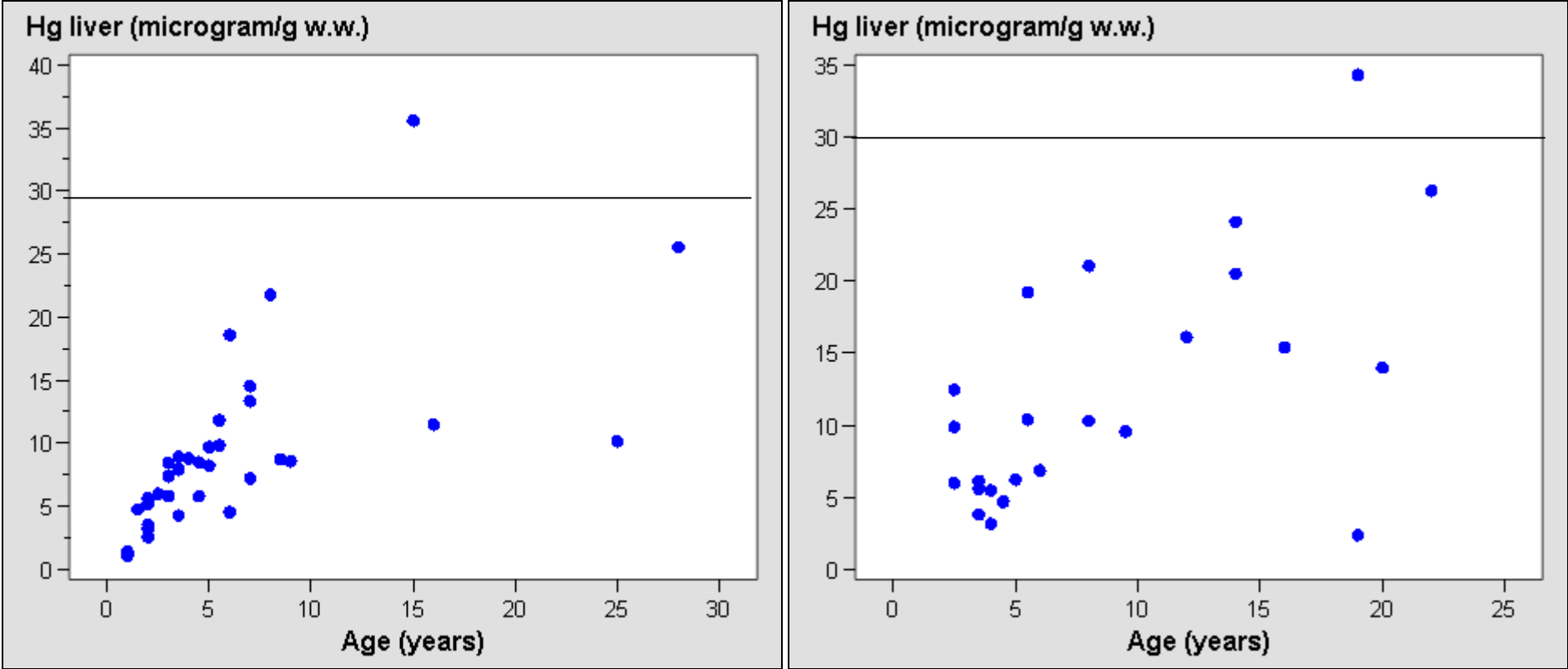
498 **Figure 2.** Kidney levels of mercury ($\mu\text{g/g w.w.}$) in 32 male (left) and 25 female (right) East
499 Greenland polar bears. Known threshold level for mercury toxicity in wildlife is given based on
500 Dietz et al., (1998a) and AMAP (2005).

501

502 **Figure 3.** Mercury concentration vs. age divided on histological lesions (yes: square; no: triangle).
503 Visible Ito cells=IC; portal bile duct proliferation and fibrosis=PB/F; lipid granulomas=LG and
504 proximal convoluted tubular hyalinisation=TH.

505 FIGURES

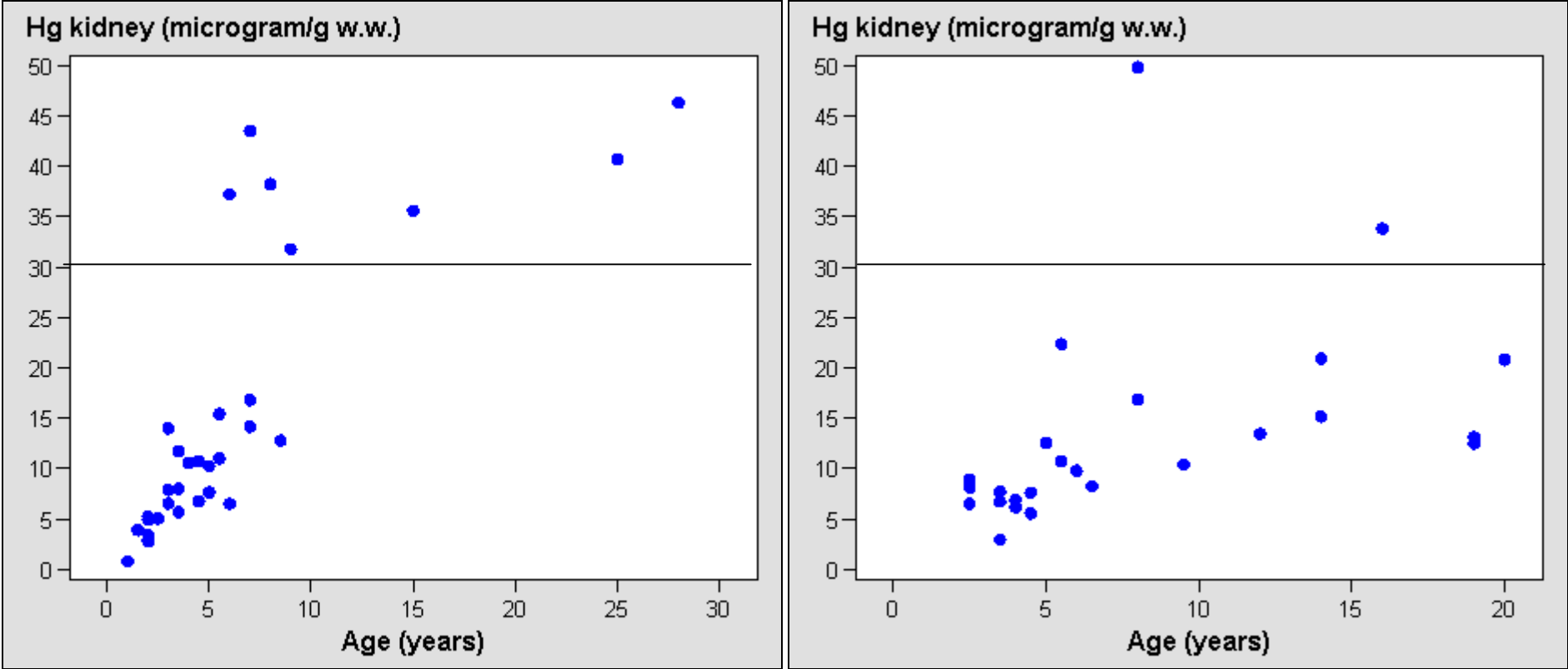
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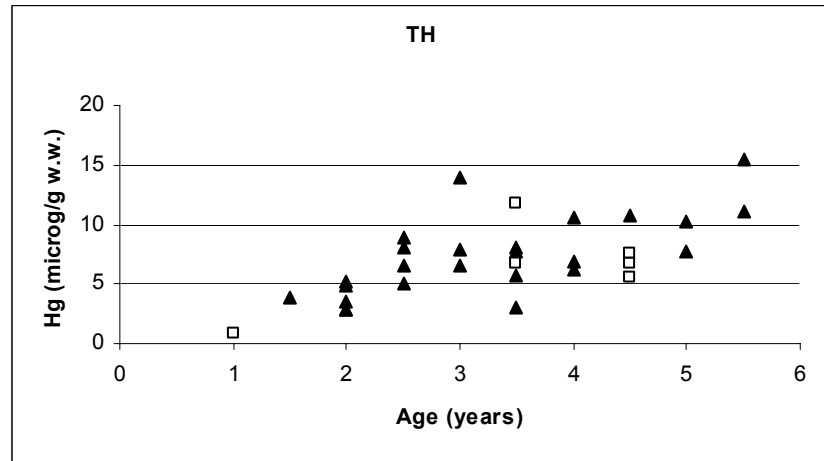
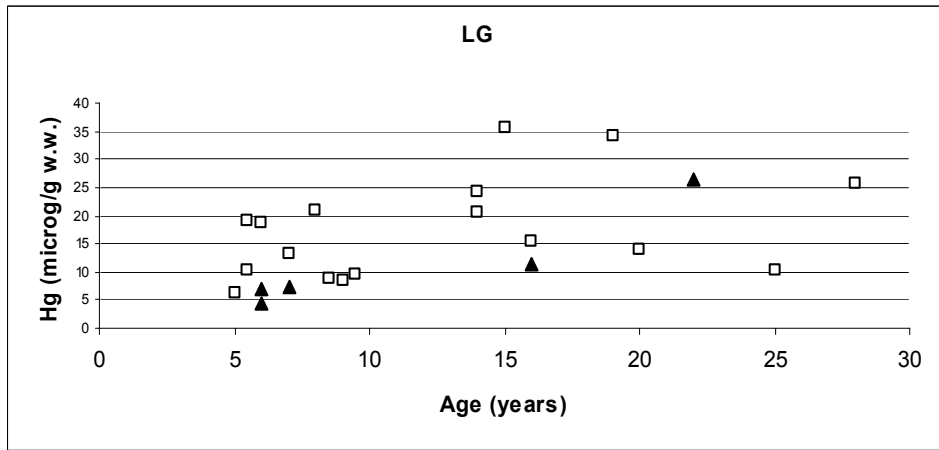
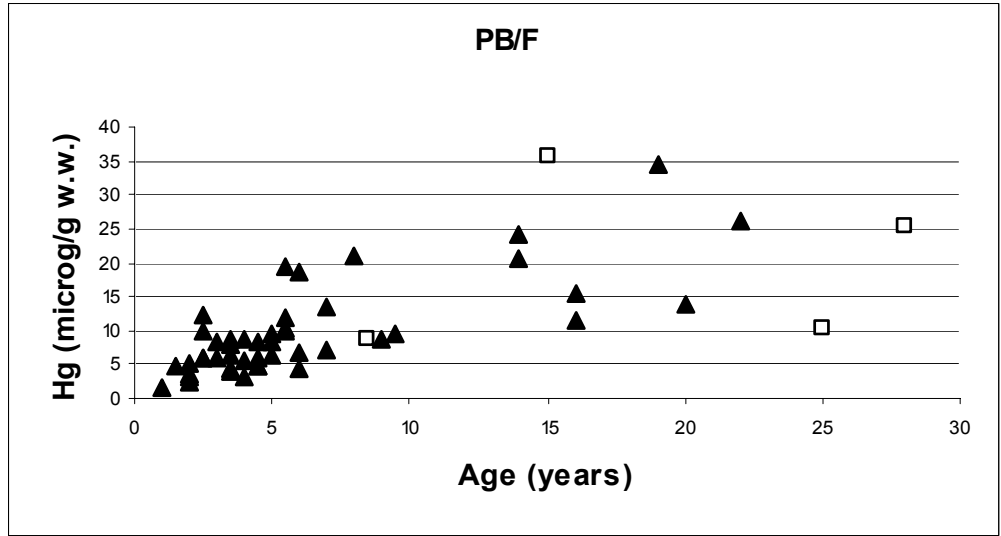
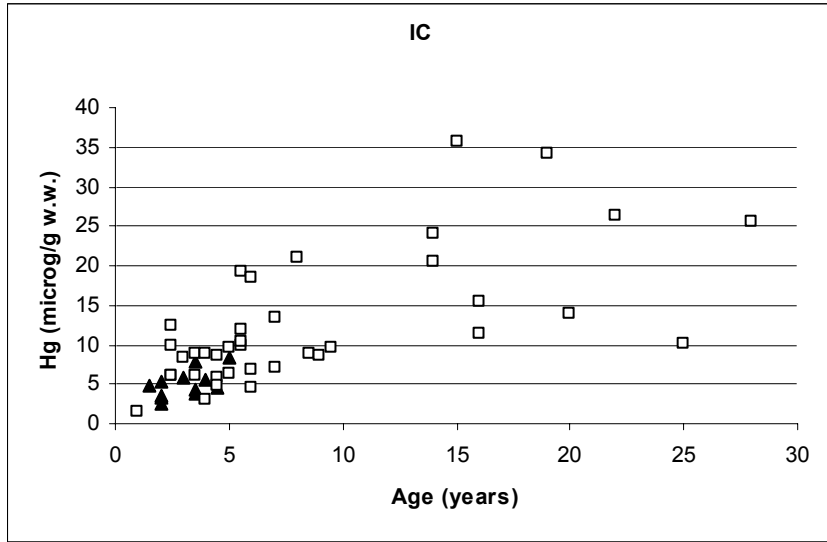
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508

FIGURE 1



511 **FIGURE 2**



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513

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FIGURE 3