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Greenland sledge dogs (*Canis familiaris*) develop liver lesions when exposed to a chronic and dietary low dose of an environmental organohalogen cocktail

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Abstract

We assessed the relationship between exposure to organohalogen polluted minke whale (*Balaenoptera acutorostrata*) blubber and liver morphology and function in a generational controlled study of 28 Greenland sledge dogs (*Canis familiaris*). The prevalence of portal fibrosis, mild bile duct hyperplasia, and vascular leukocyte infiltrations was significantly higher in the exposed group (all Chi-square: $p<0.05$). In case of granulomas, the frequency was significantly highest in the bitches (P generation) while the prevalence of portal fibrosis was highest in the F generation (pups) (both Chi-square: $p<0.05$). No significant difference between exposed and controls was found for bile acid, ALAT, and ALKP, while ASAT and LDH were significantly highest in the control group (both ANOVA: $p<0.05$). We therefore suggest that a daily intake of 50–200 g environmentally organohalogen polluted minke whale blubber can cause liver lesions in Greenland sledge dogs. It is reasonable to infer that other apex predators such as polar bears (*Ursus maritimus*) and humans may suffer from similar impacts.

Keywords: Alanine aminotransferase (ALAT); Alkaline phosphatase (ALKP); Aspartate aminotransferase (ASAT); *Balaenoptera acutorostrata*; Bile acid; Blubber; *Canis familiaris*; Chlordanes; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; HCB; Hexacyclohexanes (HCHs); Histopathology; Liver; Lactate dehydrogenase; LHD; Mercury (Hg); Minke whale; Organohalogen contaminants; Polar bear; Polybrominated diphenyl ethers (PBDEs); Polychlorinated biphenyls (PCBs); Sledge dogs; *Ursus maritimus*; West Greenland

1. Introduction

As other mammalian top predators in the Arctic such as Arctic foxes (*Alopex lagopus*) and polar bears (*Ursus maritimus*), Greenland sledge dogs (*Canis familiaris*) rely on marine mammals as a food source (Born, 1983; Sonne et al., 2006a). Marine mammals in East Greenland, Svalbard and the Kara Sea carry the highest concentrations of long-range transported anthropogenic toxic and xenoendocrine disrupting organohalogen compounds (OHCs) such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and polybrominated diphenylethers (PBDEs) (AMAP, 2004). OHCs are suspected to have various impacts on endocrine (Braathen et al., 2004; Haave et al., 2003; Oskam et al., 2003, 2004; Skaare et al., 2001), immunological (Bernhoft et al., 2000; Lie et al., 2004, 2005), reproductive (Wiig et al., 1998; Sonne et al., 2006b), bone (Sonne et al., 2004), and internal organ morphology (Sonne et al., 2005, 2006c) markers in

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polar bears. The impact on the immune system and kidneys has recently been confirmed in a thorough controlled generations study on Greenland sledge dogs exposed to polluted minke whale (*Balaenoptera acutorostrata*) blubber containing similar concentrations as the ringed seal (*Phoca hispida*) blubber ingested by the polar bears (Sonne et al., 2004, 2006c, 2007a, b, in press-a, b).

No controlled studies have tried to link chronic exposure to OHCs and liver lesions while several studies have investigated the acute and lethal impacts. In rats and mink, several acute studies of PCBs have associated these compounds with hepatotoxicity (Bergman et al., 1992; Bruckner et al., 1974; Chu et al., 1994; Jonsson et al., 1981; Kelly, 1993; Kimbrough et al., 1971; MacLachlan and Cullen, 1995; Parkinson, 1996). Specifically in the liver, acute OHC toxicity is mediated through subcellular toxicity leading to impaired ATP, protein synthesis, etc. (Kelly, 1993; Parkinson, 1996), while chronic exposure also may affect endocrine homeostasis via upregulation of CYP-isozymes (e.g. 1A and 1B) (Boon et al., 1992; Lin et al., 2003; van Duursen et al., 2003; Wong et al., 1992).

In the Arctic, a link between OHC pollution and the prevalence of liver lesions has only been reported in polar bears from East Greenland (Sonne et al., 2005). The investigation had a robust cohort (32 subadults, 24 adult females, and 23 adult males) as well as age grouping (1–28 years) and examined the relationship between environmental OHC exposure (cocktail) and liver morhphlogy. However, no control group (unexposed wild polar bears) was available and therefore no final cause and effect conclusions was drawn. Beside this investigation, environmental studies of OHCs and liver morphology has only been conducted in birds, such as cormorants (*Phalacrocorax carbo*) (Fabczak et al., 2000), and fish, such as common bream (*Abramis brama*) (Koponen et al., 2001). We therefore decided to investigate the implications from dietary chronic OHC exposure on liver morphology and function in arctic top predators under controlled conditions. That was facilitated via a generational study including a control and an exposed group of West Greenland sledge dogs. The exposed group was fed minke whale blubber from a single individual that had high levels of various OHCs as well as being nutritionally rich in n-3 fatty acids, whereas the control group was fed a diet of pork fat which allowed the exposed dogs to serve as a surrogate model for polar bears. We here present the liver histopathology and haematological clinical-chemical endpoint markers from the 28 sledge dogs included in the study.

2. Materials and methods

2.1. Experimental design

The captive sledge dog experiment was performed on a licence granted by the Home Rule Government in Greenland as part of a larger OHC study. The experimental design was conducted as a randomized blind intervention study on West Greenland sledge dogs in Aasiaat, Disco Bay, West Greenland, under conditions of contaminant exposure via the consumption of a natural diet, and taking seasonal fasting from yearly climatic oscillations and environmental factors such as infectious agents (micro pathogens) into account. The parent bitch generation (P) was composed of pair-wise sisters randomly divided into eight exposed and six control individuals in order to avoid the influence from age, sex, and genetics (Table 1). The bitches had 14 pups (9 controls and 5 exposed). Furthermore, all bitches were mated with the same 5-year-old male sledge dog. The exposed group was fed 50–200 g/day (equivalent of 2.9–7.0 g/kg) of OHC contaminated, West Greenland minke whale blubber rich in polyunsaturated lipids. The control group was likewise fed 50–200 g of pork fat per day (relatively low in both OHCs, mercury, and polyunsaturated fatty acids compared to the exposure cohort) aiming at harmonizing the energy intake by attaining comparable weight among sisters and brother in the two groups (Table 2). The bitches were fed exposed and control food, respectively, immediately after entering the project at age 2 months, while pups were fed exposed and control food after weaning at age 6–8 weeks. All dogs were kept in the locally required chains, which also served to control food intake. The dogs were regularly exercised and examined by a field veterinarian. The dogs were also immunized for canine distemper virus, parvovirus, hepatitis virus, parainfluenza virus, and rabies (Duramune 4 Vet Scanvet®). They were fed equal amounts of standardized Royal Canin Energy 4300/4800 dry dog pellets (50–200 g/day) to cover basic nutrients and microelements (http://www.royalcanin.com/) (Table 2). The aim of the study was to determine effects on liver morphology and function between the two

Table 1
Data on the 14 bitches (P generation) and their 14 pups (F1 generation) at time of blood and liver tissue sampling

Individual	Group	Sex	Age (days)	BLCCP
Pup	C	M	24	N
	C	M	24	N
	C	F	75	N
	E	M	336	N
	E	M	336	N
	E	M	336	Y
	E	F	336	Y
	E	M	336	Y
	C	F	357	Y
	C	M	357	N
	C	M	357	Y
	C	M	357	Y
	C	M	357	Y
	C	F	357	Y
Bitch	E	F	381	Y
	C	F	381	Y
	E	F	404	Y
	C	F	404	Y
	E	F	500	Y
	C	F	500	Y
	E	F	556	Y
	E	F	556	Y
	C	F	556	Y
	E	F	635	N
	C	F	635	Y
	E	F	636	Y
	C	F	669	Y
	E	F	669	N

C: Control (fat); E: exposed (blubber); F: female; M: male; BLCCP: blood liver clinical-chemical parameters; Y: yes; N: no.