POTENTIAL BIOMARKERS FOR ASSESSING THE EXPOSURE AND EFFECTS OF THE CONTAMINANT LOAD IN BALTIC RINGED SEALS (*Phoca hispida*) AND GREY SEALS (*Halichoerus grypus*)

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Introduction

The Baltic Sea is one of the most polluted water basins in the world [1,2]. The Baltic seals have been suffering from a heavy contaminant load consisting mainly of chlorinated hydrocarbon contaminants and heavy metals [1,3,4,5,6]. Pollutant levels have decreased since the peak in the 1970s, but are still high enough to cause a threat to the aquatic ecosystem [7].

The level and type of contamination in organisms is dependent on the availability, persistence, accumulation and metabolism of the compound. The Baltic seals are especially vulnerable to contaminant exposure as they have a lower detoxification capacity than terrestrial animals, and as their large lipid reserved serve as deposits for lipophilic compounds [8]. A complex of pathological disorders has been described in the Baltic seals from samples obtained during the 1970s and 80s. This disease complex includes uterine occlusions, ulcers, adrenocortical hyperplasia, renal failure, uterine tumours and skull lesions [3,4,9,10]. The pathological changes have been associated with the heavy pollution load, but the mechanism linking them together is not known.

Biological markers (biomarkers) that reflect the exposure levels and/or health status of wildlife populations can be used to assess the impact of the total pollution load. Biomarkers have been developed for monitoring exposure, effects and susceptibility of xenobiotic stress. In this study, we used cytochrome P450 (CYP) enzymes, vitamin A, vitamin E and haematological and blood chemistry parameters to screen for potential biomarkers of exposure and effect of the Baltic contaminant load on ringed seals (*Phoca hispida*) and grey seals (*Halichoerus grypus*). Samples from ringed seals from Svalbard in the Arctic and from grey seals from Sable Island in Canada were used as reference material in terms of the pollution load. In these areas, average SPCB and SDDT levels of less than 3 mg/kg (lipid weight) in blubber have been reported [11,12].

Methods and Materials

Baltic ringed and grey seals were sampled in the Bothnian Bay, the Baltic Sea and reference samples were collected from ringed seals from Svalbard and grey seals from Sable Island during 1996-1998. All seal populations were sampled during their moulting season at approximately the

same phase of their annual reproductive cycle. Sampling, preparation and storage of samples has been described in detail elsewhere [13, 14, partly unpublished]. Age was determinated by counting the annual layers of a canine tooth [15], and the condition was estimated by a condition index determined by the blubber thickness at sternum in relation to the body length [16,17].

Extraction and analysis methods of PHAHs (DDTs and PCBs) from liver are described in detail in a manuscript under preparation by Koistinen et al. 34 PCB congeners and both o,p' and p,p'-isomers of DDT, DDE and DDD were determined. PCB and DDT results are presented on lipid weight basis. For this study, mercury (Hg), Selenium (Se), Cadmium (Cd) and lead (Pb) were determined. Metal extraction and analysis methods have been described previously [14]. Metal concentrations are presented on a fresh weight basis.

Sampling, extraction and analysis of vitamins, haematological and blood chemistry parameters and cytochrome P450 enzymes have been reported in detail [13,Party unpublished]. Total vitamin A concentrations in liver and blubber represent the sum of retinol and retinyl palmitate, while retinol levels were used in plasma. α-Tocopherol represents the total vitamin E concentration in all tissues studied. For the statistical analyses, the data were divided into four groups according to species and geographical location.

Results and Discussion

Levels of all DDT compounds, PCBs and mercury were clearly elevated in both Baltic seal species compared to seals from the reference areas, while cadmium showed the opposite geographical trend (Table 1). The clearly higher SPCB and SDDT burden in the Baltic ringed seals compared to grey seals could be caused by differences in diets and feeding activity, and/or species specific physical (body size) and physiological (metabolic capacity) characteristics. PCB profiles showed relatively higher proportions of the lower chlorinated congeners in the reference areas, while the more persistent congeners were more prominent in the Baltic populations. This could be explained by the Baltic seals having a better metabolising capacity of the less chlorinated congeners due to the induced CYP enzyme levels. The tissue distribution and accumulation pattern of all metals showed a similar trend in the two seal species, mercury being concentrated to the liver and Cd to the kidney. As the central part of the detoxification mechanisms of mercury is the formation of a stabile mercury-selenium complex, a Hg: Se ratio close to one in ringed seals indicates that they are capable of detoxifying the hepatic mercury load. The elevated Hg: Se ratios in all grey seals suggest that they are suffering from a mercury load they are not capable to detoxify with selenium.

All PCBs and the DDT compounds showed a very consistent correlation matrix with each other and with the physiological parameters in ringed seals, indicating that all determined compounds are of toxicological significance. When summarising the observed relations within and between groups of biomarkers and contaminants, a clear and species specific pattern was revealed. PCBs, DDTs, EROD, vitamin E, alkaline phosphatase and the mercury-selenium complex formed one group in ringed seals. In grey seals, the picture was more complex. PCBs, DDTs, EROD, vitamin E, plasma vitamin A and phosphorus were connected

in one group with an opposing group consisting of liver and blubber vitamin A, Cl and Cd. The connection of organic contaminant levels to EROD and vitamin E in ringed seals could be explained by a dioxin like induction of CYP1A via the Ah-receptor, resulting in an increased need for radical scavengers such as vitamin E. An increase of AFOS levels in chicken females after *in ovo* exposure of DDT has been reported [18], suggesting an estrogenic effect of DDT. Further research is needed to confirm the possible estrogenic effects of the contaminants in the Baltic ringed seals. A similar pattern between the organic contaminant load and EROD and vitamin E was seen in grey seals as described above. The reduced vitamin A levels in the liver and blubber could be explained by a toxic effect of PCB [19,20]. The simultaneous elevation of phosphorus and calcium (Ca) levels in the Baltic grey seals could be due to an increased binding of excess Ca to phosphorus in the blood, suggests an increased mobilisation of Ca from the skeleton [21]. This hypothesis is supported by previous reports linking an elevated occurrence of skull bone lesions in the Baltic grey seals to the high load of organic pollutants [10]. Further studies are needed to demonstrate possible toxic effects of organic pollutants on bone turnover in seals.

With biomarkers it is possible to observe the subleathal responses possibly affecting the physiological balance that ultimately leads to pathological impairments and diseases. Of the potential biomarkers in this study, EROD and vitamin E could be used as exposure biomarkers for organic pollutants in both species. The possibility of using the induction of AFOS in ringed seals and phosphorus in grey seals as effect biomarkers of the organochlorine load requires a better understanding of the mechanistic connection between the contaminants and physiological parameters. In grey seals, reduced hepatic or blubber vitamin A levels could be used as effect biomarkers for DDT exposure.

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Table 1. Sample size, SPCB, SDDT, heavy metal and selenium concentrations in ringed and grey seals from the Baltic Sea, Svalbard and Canada. Results are presented as means ± SD (range). The significance of geographic variation is presented as *$p < 0.01$. Significant species difference within the Baltic Sea or between the two reference areas is marked $^{\#}p < 0.01$.

	Ringed seal		Grey seal	
Parameter (unit)	Baltic Sea	Svalbard	Baltic Sea	Sable Island
Sample size (n)	27-29	26-28	29-30	19-20
SPCB [a] (μg/g l.w.)	66 (17-152)*	1.1 (0.3-8.8)$^{\#}$	28 (10-74)$^{\#}$	8.2 (0.3-58)*
SDDT [a] (μg/g l.w.)	38 (10-101)*	0.4 (0.2-1.7)$^{\#}$	7.6 (3.3-14)$^{\#\#}$	1.8 (0.3-4.5)*
Mercury [a, b] (μg/g f.w.)	71 (8.2-124)*	1.2 (0.4-2.6)$^{\#}$	78 (15-348)	109 (27-278)
Selenium [a, b] (μg/g f.w.)	20 (3.2-35)*	2.5 (1.5-3.2)$^{\#}$	20 (2.7-79)	28 (9.3-83)
Cadmium [a, b] (μg/g f.w.)	0.7 (0.3-0.9)*	4.6 (0.3-11.8)	0.4 (0.2-1.1)	1.8 (0.4-4.1)*
Lead [a, b] (μg/g f.w.)	(n.d.-0.25)	(n.d.-0.15)	(n.d.-0.1)	(n.d.-0.1)

l.w., lipid weight
f.w., fresh weight
n.d., no detection
Results have been presented previously in [a] Koistinen et al. (2000) and [b] Fant et al. (2000)