POP load and vitamins as potential biomarkers in the Baltic seals

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Introduction

Exceptionally high levels of polychlorinated biphenyls (PCB) and 1,1,1.trichloro-2,2-bis[p-chlorophenyl]ethane (DDT) and its metabolites were reported in the Baltic seals in the late 1960s and early 1970s [1]. PCB levels in ringed seals, in particular, are still high enough to threaten the well being of the animals. The observed difference in contaminant pattern between ringed and grey seals in the Baltic has not been explained, but could be partly due to species-specific food sources.

Several pathological and biochemical changes observed in the Baltic seals correlate with the individual POP loads [2,3]. Of the observed biochemical changes, elevated cytochrome P4501A (CYP1A) levels, decreased liver vitamin A stores and increased vitamin E levels in blubber or plasma, have been proposed as possible biomarkers of contaminant load in Baltic seals [3]. However, as the vitamin A and E status of marine mammals also reflects the nutritional vitamin level, the lower vitamin A and elevated vitamin E levels observed in the Baltic seals could be a reflection of the levels of these vitamins in their food sources.

The aim of this study was to investigate the contaminant load in the Baltic seals and to evaluate the utility level of potential exposure and effect biomarkers. Seals from less contaminated areas were used as reference material (Svalbard and Sable Island, Canada). In the present study, POP and vitamin levels were also studied in seal prey species in order to study the transfer of these compounds to grey and ringed seals from their main food sources

Materials and methods

Sample collection and preparation: **Samples of seals and their main species of prey were collected for POP and biochemical analyses. Prey species of seals were collected from the Baltic and reference areas and analysed for vitamins and POPs to study the transfer of vitamins and POPs to seals. Samples of Baltic seal prey species were caught in the Bay of Bothnia, in July 2001. The Svalbard ringed seals prey species were caught in late May 2001. The main prey species of the grey seal population at Sable Island were sampled in February 2002. Detailed descriptions of the sampling procedure and the condition of the Baltic and reference seals and prey species are reported elsewhere [3,4]. Liver, blubber and blood samples were obtained from 10 to 30 seals per population during their moulting season at approximately the same phase of their annual reproductive cycle.**

Chemicals: **Baltic prey and seal species were analysed for 32 PCB congeners (referred to SPCB including non-*ortho* and mono-*ortho* PCBs: IUPAC nos 33, 60, 66, 74, 105, 114, 118, 122, 123, 156, 157, 167, 189) and di-*ortho* PCBs: IUPAC nos 18, 47, 49, 51, 52, 99, 101, 110, 128, 138, 141, 153, 170, 180, 183, 187, 194, 206 and 209) and the *o,p'*- and *p,p'*-isomers of DDT, DDE (1,1-dichloro-2,2-bis (chlorophenyl) ethylene) and DDD (1,1-dichloro-2,2-bis (p-chlorophenyl) ethane). Whole fish or fish muscle tissues and seal liver tissue were used or the contaminant analyses [4,5]. Methods are described elsewhere [4,5]. The contaminant levels in the individual prey species were added together according to their relative representation in the springtime diet of each seal species (Stenman & Poyhonen, unpublished) [6,7].**

Vitamins: **Seal liver samples and pools of whole prey species were used in the vitamin analyses. The fat-soluble vitamins A and E were extracted according to Murk et al. and Nyman et al. [3,8], with some modifications. In short, samples were homogenized in Tris-HCl –buffer. Homogenate was mixed with diisopropyl ether (DIPE) and methanol, containing the internal standards. The organic layer was collected after mixing and centrifuging, washed in DIPE, and filtered and evaporated under nitrogen flow. The residues were dissolved in methanol-ethyl acetate. The vitamins were separated and quantified by high-performance liquid chromatography (HPLC), using a Waters Symmetry C18 (5 um) or a Waters Nova-Pak C18 reverse-phased**

column and guard column. Samples were analysed by running 93 % methanol, followed 99 % methanol. As retinol could not be extracted from the herring samples using the method described above, a saponification method developed by Ollilainen et al. [9] was used with some modifications. In short, fish were mixed with water and ethanol and ascorbic acid. After mixtures were hydrolysed with 50 % potassium hydroxide. The vitamin-containing portion were extracted with petrol ether and diethyl ether, washed free of alkali, dried and dissolved in hexane. The vitamins were separated and quantified by HPLC using Li Chro CART Si-60 (5 um). The samples were eluted with 99.5 % hexane and 0.5 % 2-propanol. Vitamin A compounds were detected using a Waters 486 Tunable Absorbance UV-Detector and vitamin E compounds using a Shimadzu RF-535 Fluorescence HPLC Monitor. Vitamin A and E analyses of seal samples are described elsewhere [3]. We used liver retinyl palmitate for the vitamin A level, and blubber rac-5,7-dimethyltocol for the vitamin E level in this study. The vitamin levels in the prey species were added together according to their composition in the springtime diet of each seal species as described in section 2.1.

Results and discussion

Chemical data:**.Hepatic SPCB and SDDT levels for Baltic seals are presented in Table 1. The levels of both SPCB and SDDT were higher in the Baltic ringed seal compared to grey seal. A comparison of the pollutant levels in the Baltic seals and their diet shows that ringed seals are ingesting more PCB and DDT compounds than grey seals, and this corresponds to elevated levels of contaminants in their tissue (Figure 1). The analysis of the POP load in the Baltic seals and in their diet revealed certain differences. There were higher percentages of non-ortho and mono-ortho PCBs in the diet than in the seal tissue .The metabolite p,p'-DDE represented the main proportion of the total DDT load in both the diet and the seals. The diet also contained approximately on fourth of unmetabolised DDT, whereas this is almost completely metabolised in the seals.**

The bioaccumulation for SPCB was greater in ringed seals than in grey seals (Figure 1), which indicates that grey seals have either less capacity for metabolising these compounds, or that the metabolism is less efficient at lower exposure level. Comparison between PCB groups show that both species of seal accumulate less non-ortho and mono-ortho PCBs than di-ortho PCBs,

which could be explained by highly elevated CYP1A expression, as CYP1A is mainly associated with non- and mono-ortho PCBs [10]. The levels of SDDT and its components in the seals compared to their food showed no difference between the two Baltic seal species (Figure 1). The higher level of SDDT in ringed seals compared to grey seals could thus be explained by the differences in their diets. The accumulation for SDDT was greater than for SPCB in both ringed seals and grey seals, which suggests that DDT compounds persist longer than PCBs.

Table 1. SPCB, SDDT in seal liver (ug/g lipid weight) and vitamin A and E in seal liver or blubber (ug/g fresh weight). Significance of species (POPs) or geographical (vitamins) difference is marked with * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

	Ringed seal		Grey seal	
	Baltic	Svalbard	Baltic	Sable Island, Canada
n [a]	26	29	20	20
age [a]	11 ±6	8 ±6	13 ±7	19 ±7
male [a]	9	12	9	10
female [a]	17	17		10
SPCB [a]	34 ±22		59 ±40***	
SDDT [a]	59 ±39		85 ±34***	
vitamin A, liver [b]	438 ±233***	1877 ±1350	137 ±61***	509 ±305
vitamin E, blubber [b]	219 ±75***	68 ±42	195 ±73***	58 ±29

[a] Nyman et al., 2002
[b] Nyman et al. 2003

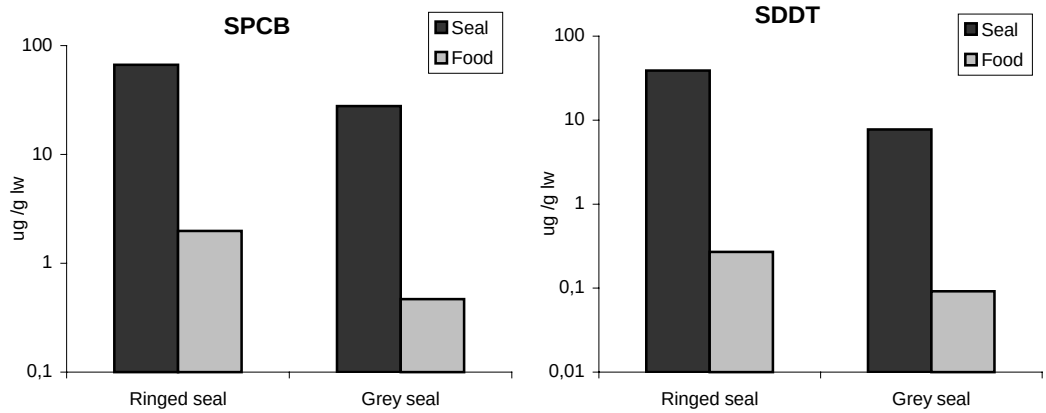

Figure 1. Levels of SPCB and SDDT (ug / g lipid weight) in the Baltic seals compared to their food.

Vitamins: **It is probable that none of the seal populations suffer from vitamin A deficiency due to vitamin A deprivation [11]. The levels of vitamin A in the seals compared to their food was in the same range in the Baltic seals and the Canadian grey seals, but approximately 5-7 times higher in the ringed seals from Svalbard (Figure 2). However, it is not known whether vitamin A is stored in the body up to a certain limit or according to supply. Consequently, the low levels of stored vitamin A in the Baltic seals could indicate either a toxic effect on vitamin A dynamics caused by the high POP load in both species, or a toxic effect that is seen only ringed seals while the grey seal dietary levels are responsible for the tissue vitamin A levels.**

Vitamin E levels in the Baltic grey seal and Svalbard ringed seal diet were less than half of the recommendation for northern fur seals [11]. Nevertheless, requirements might vary between species living in different climates, and dietary vitamin levels may vary seasonally. The accumulation for vitamin E was higher in both Baltic seal populations than in the reference seal populations (Figure 2). Thus, the elevated vitamin E status in the Baltic seals could be a response to an increased requirement, and thus increased ingestion, of vitamin E [12] for protection against the oxidative response [13] caused by their high POP load [14].

In conclusion, these results further support our previous hypothesis that the toxic effects of environmental contaminants could be causing the observed divergence in vitamin levels between the Baltic seals and the reference seal populations, and that vitamins are potential biomarkers for contaminant load and effect for the Baltic ringed and grey seal. However, as the vitamin A

accumulation in the seal is poorly known, more research should be done on the vitamin A dynamic in order to better understand the influence of contaminants on stored vitamin A.

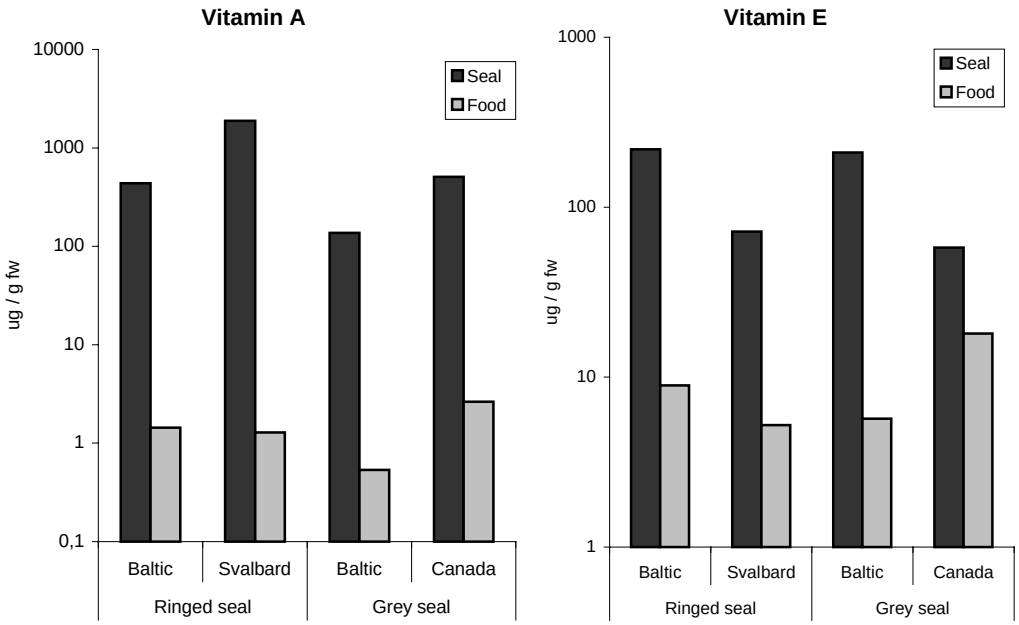

Figure 2. Levels of vitamin A and E (ug / g fresh weight) in the Baltic and reference seals compared to their food.

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