Variation of the composition of PCB mixtures in blubber of the harbour seal (Phoca vitulina) with sex, age and location

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Introduction

Many diseases occurring in seals, e.g. skull deformations, hyperplasia of the adrenal zona fasciculata and uterine occlusions in the Baltic Sea populations, and phocine distemper virus infection in North and Baltic Sea populations have been related to environmental contamination. It is still difficult to estimate the hazard associated with high body burdens of these contaminants. Missing data and small numbers of animals are the main reasons for highly variable and in many cases contradictionary results in epidemiological studies. The relevance for toxicity of single compounds or the simultaneous presence of compounds in complex mixtures of environmental contaminants is not known. Most congener specific evaluations of PCB-mixtures were performed to compare the composition of PCBs between species or within the food chain in order to estimate the ability of the species to metabolize the congeners (14,1). Comparing mixtures within a species has been dedicated to the differential transplacental or lactational transfer of congeners from mother to fetus, newborn or suckling pups (4,8). There is very limited informa-tion available about the variability of the composition of the mixture within species and parame-ters that might have an influence like ΣPCB, age and sex (11,6). The aim of the study was to further elucidate this problem in order to get helpful information for epidemiological studies.

Materials and Methods

Blubber from 31 seals, found dead on the North Sea coast of Schleswig-Holstein, Germany, during the epizootic disease of 1988/9, including 20 animals from a previous study (11). Furthermore blubber of ten healthy harbour seals from Iceland, shot in the summers of 1988 and 1989, and biopsies of blubber of a group of 12 seals fed with Atlantic fish from the seal orphanage in Norden, Norddeich, Germany, were analysed. For further data see *table* of results. The material was processed and determination of PCBs (51 congeners) was carried out as described previously (11,10,5,2). In some samples **146** could only be detected together with **132** (11); therefore, the number of animals contributing to the groups changed as follows: North Sea female adults: n=6, male adults: n=9.

$$G = \sum_{i=1}^{51} (e_i - t_i) \cdot a_i$$

e_i = ratio of the congener in mol-% in the analysed PCB-mixture, t_i = ratio of the congener in mol-% in the PCB-mixture used as standard, a_i = degree of chlorination of the congener i

The degree of metabolism (G) is a simple parameter to describe a complex PCB-mixture (7). The higher G is the more the mixture is changed by metabolism. Comparison between groups was carried out using the Mann, Whitney and Wilcoxon U-test for independent non-parametric data and Spearman rangcorrelation, $\alpha=0.05$, was applied.

Results

A positive correlation between ΣPCB and age was found in male seals from the North Sea. In female North Sea and Icelandic seals negative correlation coefficients (non significant) were

found. Furthermore in male North Sea seals there was a positive correlation between ΣPCB as well as age and degree of metabolism. Also in seals from Norddeich ΣPCB were strongly positively correlated with the degree of metabolism.

	age [year]	ΣPCB [mg/kg]	G		age [year]	ΣPCB [mg/kg]	G
ICELAND				**NORTH SEA**			
young	0	1.6	0.051	**young**	0,0,0,1,1,		
	1	5.4	0.271	n=9	2,2,2,2		
adult **male**	13	8.0	0.484	*median*		31.6	0.511
adult **female**	4,4,5,11,			*mini*		5.7	0.186
n=7	12,13,13			*maxi*		180.3	0.606
median		3.0	0.363	adult **female**	4,4,4,4,5,6, 6,		
mini		1.2	0.180	n=11	8,8,18,26		
maxi		4.7	0.534	*median*		64.7	0.584
				mini		7.6	0.419
NORDDEICH				*maxi*		192.6	0.816
young	all 1			adult **male**	6,6,7,7,9,9,11,		
n=12				n=11	13,14,15,28		
median		5.8	0.307	*median*		139.6	0.643
mini		2.1	0.016	*mini*		68.7	0.591
maxi		19.1	0.680	*maxi*		247.1	0.752

Table: The medians, minimal (mini) and maximal (maxi) values of the ΣPCB concentrations [mg/kg] based on extractable lipids in blubber and of the degree of metabolism (G) of the groups of seals are presented.

Congener specific results:

Congeners **17**, **32**, **33** and **41** were detectable only in a very small number of samples (**18**, **22**, **42** only in Norddeich) and were therefore excluded from further consideration.

Correlations between mol% of congeners and ΣPCB and/or age:

Norddeich: *positive* correlation with ΣPCB **199**, **203**, (**194**, **195** not in the mixture), **180**, **187**, (**189** not in the mixture), **170**, **171**, **146**, **153**, (**167** not in the mixture), **138**, (**128** positive correlation coefficient), **99**; *negative* correlation **141**, **149**, **151**, **95**, **101**, **18**, **22**, **42**, most other congeners negative coefficients. ***young***, North Sea: *positive* correlation either with ΣPCB **99**, **153** or with age **203**, **179**, all others that increased with ΣPCB in seals from Norddeich showed a positive coefficient with age and ΣPCB **170**, **171**, **180**, **138**, (**146** only ΣPCB), (**187**, **199** only age); *negative* correlation with ΣPCB **141**, **149**, **151**, (**95**, **101** not significant) and additionally to Norddeich **28**, **74**, **66**, **118**, **136**, **177**. ***male***, North Sea: *positive* correlation with age and ΣPCB **194**, **199**, **203**, **195** (only age), **180**, **170** (only age),(**183**, **171**, **187** had positive correlation coefficients, non significant), all other congeners had *negative* coefficients, mostly significant at least in one age or ΣPCB). ***female***, North Sea and Iceland: the tendencies in both groups were the same (in Icelandic seals non significant), unless otherwise mentioned; if the correlation coefficient for age was positive then the coefficient for ΣPCB was usually negative and vice versa; *negative* correlation with age **153'**, **138**, **99**; *positive* correlation with age **199**, (**194**, **203** non significant), **180**, (**183**, **187** not in Iceland), **170**, **171**, **118**, (**28** not in Icelandic), **92**, **141** (significant in Iceland, not positive in North Sea).

Comparison of composition of mixtures within North Sea seals:

Molar ratios of **146** and **158** were higher in male than in female and young seals, of **187**, **183**, **180**, **170**, **199**, **194**, **203**, **195** male and female were higher than in young, respectively, and of most other congeners male were higher than female and young seals.

Comparison of composition of mixtures between Icelandic, Norddeich and North Sea seals:
Within the group of Icelandic seals the pup and the male seal often occupied extreme positions and in most cases that is in accordance with relationships in North Sea seals, but with **128** and **99** the molar ratios in Icelandic male were highest, while North Sea males tended to lower molar ratios. The molar ratios of many congeners differed significantly between Norddeich and Icelandic female seals on one hand, and North Sea seals on the other. Only the molar ratios of **70**, **146** and **149** were significantly higher in Norddeich seals than in Icelandic seals and of **199**, **167**, **99**, **118**, **74** and **52** vice versa. Molar ratios of Tri-, Tetra- and PentaCBs were lower in North Sea seals than in others, with a few exceptions, probably due to low concentrations and detection limits. Also Hexa- and HeptaCBs with vicinal H in m,p-position were lower in North Sea seals (exceptions **149** not in Icelandic, **179** detection limit). **156** and **158** were lower in North Sea Seals as well. North Sea seals had higher molar ratios in **153**, **146**, **138**, **187**, **171**, (**170** except young seals). The Icelandic female seals had similarly high molar ratios of OctaCBs to the North Sea seals and high ratios of the congeners **156**, **157** and **167** were determined.

Discussion

An increase of ΣPCB with age in male marine mammals is well known, also in female seals up to an age of four (15). A decrease of ΣPCB with age in female whales after reaching sexual maturity and increase as a consequence of the ceasing of sexual activity was described (12). The presented data seems to indicate the same for seals. In some species, e. g. duck, whale or Northern fur seal, EROD-activity increased with ΣPCB-concentration (13). Also changes in the mixture like the ratio **169/126** were correlated with ΣPCB. In a previous work (6) an increase of the degree of metabolism of the PCB-mixture with ΣPCB was described. That is confirmed by the additional data. In low concentrations (< 20 mg/kg) there is a high increase of G with ΣPCB that was approached by the linear regression of values of the Norddeich seals, in higher concentrations (> 50 mg/kg) the increase ceased and seemed to approch a limit. This part might be approched by the linear regressin of values of male North Sea seals. Both groups showed a strong correlation between G and ΣPCB. This relationship could be caused by the induction of enzymes, that might be supported by the findings in fur seals. Above 30 mg/kg the increase in enzyme activity ceases (13). This study shows that also within a species there are different patterns of accumulation and exretion/metabolism depending on ΣPCB, location, feed, age and sex. The obvious difference between mixtures in Icelandic seals and seals from the orphanage in Norddeich on one hand and the North Sea seals on the other hand underlines the dominant influence of ΣPCB on the mixture. The differences between them may be due to age, sex, food and sampling of probes. The seals from Norddeich were all of the same age and feeding on the same fish, therefore the main factor having an influence on the composition of the mixture was ΣPCB. The varying body burdens of the animals still remained after at least half a year in the orphanage, feeding on the low contaminated fish. Congeners with vicinal H in m,p-position as well as congeners with degree of chlorination lower than five decreased with ΣPCB (not all significantly). The mono-ortho-PCBs with five or six chlorines didn't have any tendency. Most other congeners (without vicinal H and with vicinal H in o,m-position and more than one chlorine in ortho-position) increased with ΣPCB (except **183**, **177**, **158**). Young seals from the North Sea didn't exhibit such obvious trends of congeners with ΣPCB. That might be due to varying feeding habits with age and habitat, as well as migration. In contrast to the Norddeich seals, they had negative correlation coefficients (some of them significant) in mono-ortho-PCBs. The trends of molar ratios of congeners with age and sex observed in a former study (11) could

mostly be confirmed with a higher number of animals. The high accordance of trends in the composition of the mixtures of female seals from the North Sea and from Iceland verify the importance of pregnancy and lactation for the composition of PCB-mixtures. Differences might be due to varying levels and composition of ΣPCB in fish. The trends and differences could usually be related to structural characteristics of congeners and molar ratios of congeners in fish, carefully considering the high variability (3). In order to establish temporal or spatial trends it is necessary to consider age and sex of the animals in a congener-specific evaluation otherwise results are not reliable. In spite of possibly decreasing relevance of PCBs in environmental contamination - they aren't produced any more, but there is still more than half of the world production in use - knowledge about PCBs should be taken into consideration in research on other complex mixtures such as dioxins, furans or toxaphenes; similar variability in the environment and mammals due to biological parameters can be expected. Until the relevance of mechanisms such as receptor mediated actions, single compounds or mixtures are not well understood, the interest in analytics should not be focussed on few compounds.

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