

## Composition of PCB mixtures in different organs of the harbour seal (*Phoca vitulina*)

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### Abstract

Fifty one PCB congeners (including some mono-orthos) were determined in blubber, liver, spleen and lymphnodes of twenty harbour seals (*Phoca vitulina*), which were found dead at the North Sea coast of Schleswig-Holstein, Germany, during the epizootic in 1988/9. Samples were taken from young seals (n=6; 0-2 years), adult females (n=6; 4-18 years) and adult males (n=8; 6-28 years). A positiv correlation existed when total PCB concentration ( $\Sigma$ PCB) in different examined tissues were considered. Significant differences were seen in the level of contamination between the organs and in the composition of the PCB mixtures (in mol%) both between organs and groups of seals. Within groups, tendencies related to age and/or total PCB concentration ( $\Sigma$ PCB) were observed.

### Introduction

Only very few studies carry out congener-specific analysis of different organs in marine mammals, in order to demonstrate toxico-kinetic aspects which depend on the structure and degree of chlorination of the congeners. Data established in laboratory animals may be different to marine mammals. One study determined 23 PCBs from the brain, liver, kidney and blubber of 10 male seals 1), while another compared blood, liver and blubber in three seals 2). Different methods were also developed which used the composition of PCB-mixtures to estimate enzyme activity 3) 4) 5). However, although enzyme activities were measured, these were generally not related to contamination, e. g. 6), with the exception of northern fur seals, in which a positiv correlation between  $\Sigma$ PCB and enzyme activity were described 7).

### Materials and Methods

Organ tissues of seals, found dead at the North Sea coast of Schleswig-Holstein, Germany, during the epizootic 1988/9, were obtained by the 'Institut für Haustierkunde, Universität, Kiel'. Seals were allocated to three groups according to age and sex, i) young, i. e. 0 years (n=1), 1 years (n=1), and 2 years (n=4); ii) adult femals, i. e. 4 years (n=4), 8 years (n=1), and 18 years (n=1); and iii) adult males, i. e. 6 years (n=1), 7 years (n=2), 9 years (n=2), 13, 14 and 28 years (n=1, respectively). The material was homogenized, dried with sodiumsulfate, and lipid was extracted cold using hexane/dichlormethane 80/20 (v/v). Further processing and determination of PCBs were carried out as described previously 10), with following modifications. The number of peaks was extended to 51, corresponding to congeners with IUPAC-no. **18, 17, 16/32, 31, 28,**

20/33/(21), **22**, 52/(73), 49/(43), 47/48/75, **44**, 59/42/(72/71), 41/64, 74/(61), 70/(76), 66/(80), 95/(93), 60/56, **92**, 90/101, **99**, 97/(86), 87/115, **136**, **110**, 151/(154), 135/(144), **149**, 123/118/(106), **146\***, 146/132/153, **141**, **179**, 160/138/163/164, **158**, 187/(182), **183**, **128**, **167**, 174/(181), **177**, **171**, **156**, **157**, **180**, 170/190, **199**, 203/196, **189**, **195**, **194**. Congeners are listed sequentially following their elution from the SE-54 9), which is in accordance with the used OV-73; a change in column was necessary, therefore, part of the samples were analysed using a 'HT 5' 10). Numbers printed in bold will be used to identify peaks, as their corresponding congeners represent the largest part of the peak. Congeners in brackets could not be excluded, yet their importance is questionable, as in technical mixtures their ratio in mass% is below 0,05 9). Molar ratios between organs were compared by Students-t-test for connected data. As molar ratios of congeners are not normally distributed, medians are presented in figures. Comparison between groups was carried out using the Mann, Whitney and Wilcoxon U-test from for independent non-parametric data.

\*146: This congener could only be detected together with 132 in samples analysed using the 'HT 5'; therefore, the number of animals contributing to each organ group changes as follows: i) young: lymphnodes n=1; ii) female adults: blubber n=5, lymphnodes n=0, iii) male adults: liver n=7, lymphnodes n=5.

## Results

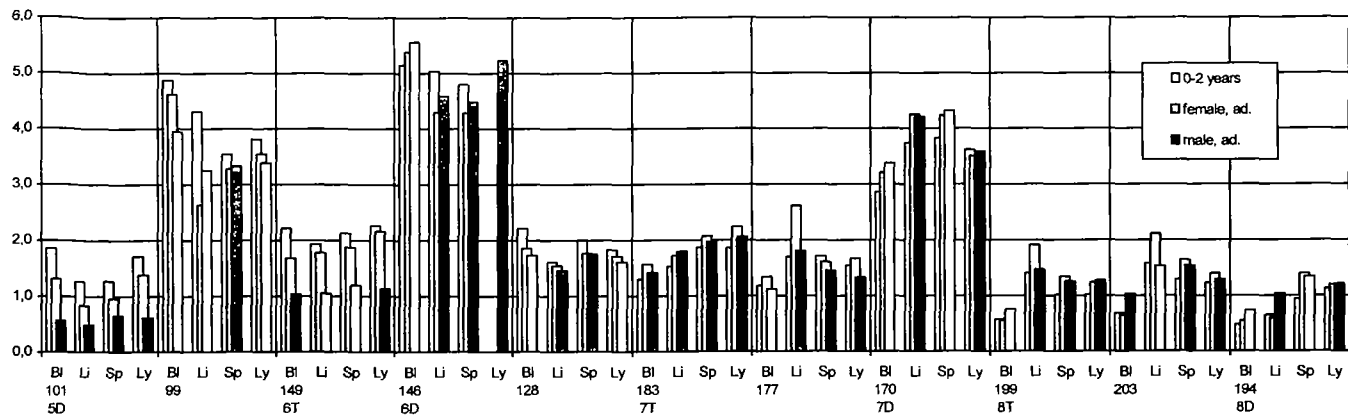
*Table:* The medians (me), minimal (mini) and maximal (maxi) values of the ΣPCB concentrations [mg/kg] based on extractable lipids in blubber (Bl), liver (Li), spleen (Sp) and lymphnodes (Ly) are presented.

	Bl			Lc			Sp			Ly		
	me	mini	maxi	me	mini	maxi	me	mini	maxi	me	mini	maxi
0-2 years	71,3	17,9	180,3	78,0	25,6	601,5	35,5	11,6	243,2	36,9	16,9	156,3
female, ad.	54,3	25,4	192,6	74,1	30,0	169,0	34,4	19,3	59,8	44,5	25,2	71,5
male, ad.	124,8	68,7	247,1	135,7	78,8	535,7	50,5	39,3	251,0	63,9	54,4	258,2
overall	94,4	17,9	247,1	112,6	25,6	601,5	48,0	11,6	251,0	55,2	16,9	258,2

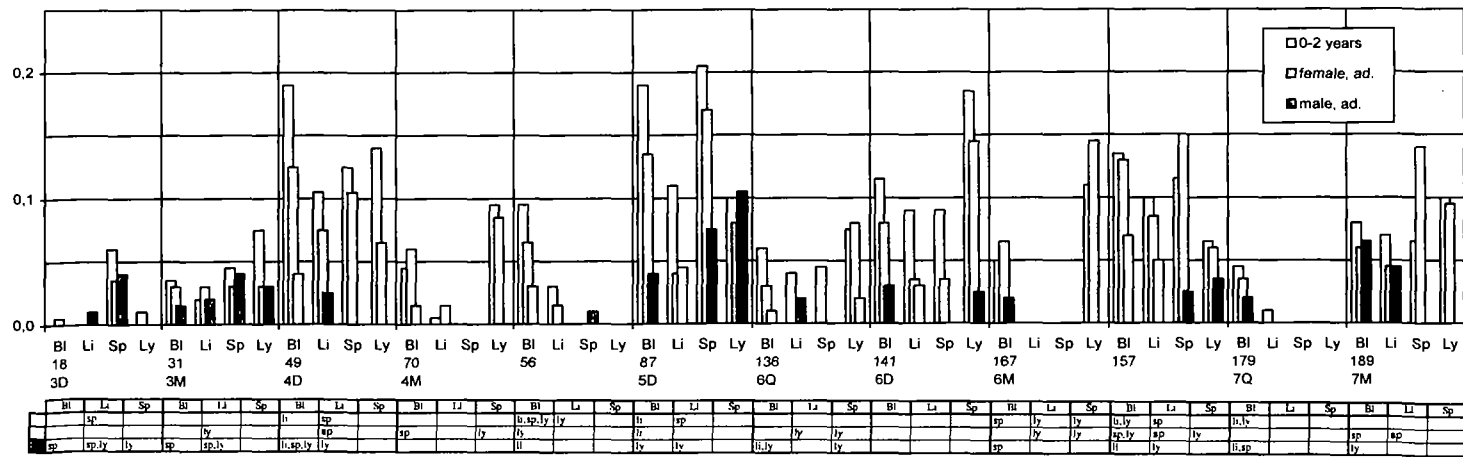
Organ total PCB concentrations are positively correlated with each other (correlation coefficient of Spearman  $r > 0,7$  in all cases/  $n=20$ ,  $\alpha=0,05$ , critical value for  $r=0,445$ ), yet differ significantly from each other ( $\alpha < 0,01$ , spleen to lymphnode  $\alpha < 0,1$ , Wilcoxon-test for connected data).

*Figure* (following pages): Medians of molar ratios in percent (x-axis) of the PCB-mixture in blubber (Bl), liver (Li), spleen (Sp) and lymphnodes (Ly) of 51 congeners (IUPAC-no.) are presented. The third lane in the text of the y-axis indicates the degree of chlorination and the number of chlorines in ortho-position of the biphenyl, M (mono), D (di), T (tri) and Q (tetra). The group of 0 to 2 year old contains 6, of adult (ad.) female also 6 and of adult male 8 individuals. 146 see text. The bar in front is printed in light grey, instead of dark grey, if the molar ratios of the males are different (U-test of Mann, Whitney and Wilcoxon,  $\alpha < 0,1$ ) in comparison to the females, white if they are different to the young and black if they are different to both. If the females and the young are different the middle grey of the bar in the middle is changed to white. The table below the bar chart lists the organs, which are different to the organ above (Students-t-test for connected data,  $\alpha < 0,05$ ), separated for the three groups.





BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	
101				149				146				128				183				177				170				199				203				194				
5D				6T				6D				7T				7D				8T				8D																



BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly	BI	Li	Sp	Ly														
18				31				49				70				56				87				136				141				167				167				157				179				189					
3D				3M				4D				4M				5D				5D				6D				6M																									

# ECOTOX II

Congeners 17, 32, 22, 42, 33, 41 and 44 were present only in a very small number of samples and therefore excluded from figures. The following points represent the main results:

1.) Congeners with 7 (not 174, 179, 189) and 8 chlorines show smaller molar ratios in the blubber than in other organs. However, in most other congeners up to hexa-CB, molar ratios in blubber are higher than liver. 2.) Molar ratios of the four octa-CBs and 180, 170, 153, 146, in blubber are higher in the male; however, ratios of almost all congeners up to hexa-CB are mostly lower in the male when compared to the young and female animals. 3.) Especially high liver ratios show female seals in the congeners 177, 199, 203, 195, 187, whereas other congeners, 138, 146, 99 and 153, demonstrate lower ratios in almost all organs, especially in liver. 4.) Due to the high variability differences between the young and females were not always significant.

Discrete data (not shown):

5.) In blubber of the male molar ratios of congeners 153, 180, 199, 203, 194 increase with contamination/age, whereas the most of the others decrease. 6.) In 2 years old and 4 years old females the molar ratios of many congeners are decreasing or increasing with  $\Sigma$ PCB. 7.) Higher molar ratios of congeners 180, 187, 183, 179, 199, 203, 194, 171, 195, and mono-orthos 156, 118, in parts 28, 74 are seen in all organs in the two older females, when compared with younger members of the group. whereas the opposite is the case with congeners 138, 99, 146 and 128. A revers situation is seen in all the mentioned congeners (not mono- orthos and 195), when compared old females. 8.) Mono-orthos in females increase with age, while the revers is the case in males.

Because of the small number of animals tendencies could not be statistically confirmed

## Discussion

The results of the study indicates that the composition of PCB-mixtures changes with the organs and depends on sex, age (and contamination). These differences were not previously seen, which may be due to the small number of animals used and the wide range of ages of male animals contributing 1) 2). In the latter study, the PCB-composition of brain differed from other organs, probably caused by the lipid composition in brain. As indicated by the first point in the results section most hepta- and all octa-CB determined are poorly or not metabolized and/or excreted in the liver in comparison to lower chlorinated congeners. Congeners mentioned in 2.) above are almost the same as in 5.) with increasing molar ratios with age/contamination. The correlation between  $\Sigma$ PCB and the composition of PCB mixtures in blubber has previously been described 4) 7), also supported by this study, 6.). Although it is known that  $\Sigma$ PCB correlate with age in the male, also supported by this study, the influence of age on the composition can not be determined with available data. Female seals show different toxico-kinetics for congeners, which can be seen in in results 3.), 7.) and 8.). These differences may be due to lactation, at least in part of the congeners, when compared with the pup; however, this does not apply to mono-ortho congeners, possibly indicating a lower capacity to metabolize the planar congeners when compared to males. This hypothesis is supported in hooded seals, where a higher EROD-activity and a stronger response to anti-P450 1A was detected in males 6).

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