Chlorinated biphenyl congeners in harbour seals (*Phoca vitulina*) and in their food. Statistical comparison of the patterns.

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1. Introduction.

Geographically separately living populations of wildlife animal species have been shown to elicit different patterns of environmental contaminants such as polychlorinated biphenyl (CB) congeners, and different animal species have also been shown to have different CB congener patterns[1,2,3]. The differences between species regarding CB congener patterns have been related to the biotransformation capacity of the species in question[1,3,4].

Polychlorinated biphenyl congeners have been measured in blubber samples from harbour seals (*Phoca vitulina*) in Denmark, after the European seal disease epidemic in 1988[2]. This study made use of seals, that were found dead at three geographically separate locations. The CB congener patterns in the samples were analyzed statistically by principal component analysis (PCA) and discriminant analysis. These analyses showed that CB congener patterns found in the harbour seals were characteristic for of each animal.

Several possible causes for the observed differences in CB congener patterns were considered. The first principal component, corresponding to the largest amount of variance in the data set analyzed, could be related to the metabolism of the CB congeners in harbour seal[2]. This interpretation was based on the results of a Dutch seal study[3,4,5,6] in which the CB congeners could be separated into persistent and metabolizable congeners.

The present study was initiated in order to obtain a better understanding of the processes underlying the results of the Danish seal study. Thus, the aim of the present study was to study the degree of reflection of the CB congener patterns of the diet (fish) in the seals.

2. Materials and methods.

The data from the dutch seal experiment in 1981-1983. The Dutch experiment took place from 1981 to 1983. Two groups of female harbour seals were held in captivity at the Institute of Forestry and Nature Research (IBN-DLO) at the island of Texel[5]. The seals were fed with fish from two different locations namely plaice (*Pleuronectes platessa*) from the Wadden Sea (the high dose group), and mackerel from the Atlantic (the low dose group). The amount of ΣCB given to the seals in the high dose group was about ten times higher than that given to the seals in the low dose group. CBs and other compounds of interest were determined in blood- and faeces samples from the seals, and results of these analyses have been published previously[3,5,6]. CB congeners were measured by capillary

gas chromatography with electron capture detection, with a medium bore SE-54 column. After an initial data screening, the CB congener data chosen for statistical analysis were (IUPAC #): 28, 52, 49, 44, 70, 101, 99, 151, 149, 153, 137, 138+163, 187, 183, 128, 177, 172, 180, 170, 201 and 194.

Statistical methods. The original data from the Dutch harbour seal study[3,6] were re-analyzed using the statistical methods used previously in the Danish seal study[2]. The statistical analyses were based on three main concepts, namely 1) normalization and standardization, 2) computation of principal components (PCs) and 3) discriminant analysis based on the PCs. The aim of the discriminant analysis was to investigate whether the variation of the PCs could be used to classify the seals according to the origin of their food and/or variation between the two different years of the experiment. The methods used for computation of the PCs and of the discriminant analysis are described previously[2]. The statistical analyses were performed for the seal samples. Fish data were normalized within each fish, and the PCs for the fish samples were computed using the means, standard deviations and PC weights obtained from the analyses of the seal samples[7].

3. Results and discussion

The first three PCs accounted for 80.3 % of the total variance in the data set. A clear separation between the different groups of samples was observed in the plot of PC3 against PC1 (Figure 1). PC2, which contributes 27 % of the variance was not related to either the experimental year or to the type of fish. Samples from seals given Wadden Sea fish are abbreviated Wadden Sea seals, and seals that were given Atlantic ocean fish are abbreviated Atlantic seals.

Variation of the CB congener pattern within the seal blood samples. No discrimination was seen between the two experimental years, within each seal group (Atlantic and Wadden Sea seal). It was possible to discriminate between the group of Wadden Sea seals and the group of Atlantic seals by use of the first and the third PC. A line separating the two groups of seal samples can be calculated from the discriminant analysis. Only a single sample out of 41 seal blood samples was misclassified in this way.

Variation of the CB congener pattern within the fish samples. The CB congener patterns of the Wadden Sea fish samples, were slightly different in the two experimental years. It is likely, that this variation is due to real differences in the fish CB congener patterns between the two years, but it may also be due to analytical variability. As this variation is minor, it was decided to classify the Wadden Sea fish sample CB congener patterns as a single group, independent of experimental year. Only a single Atlantic fish sample was present in the data. We are aware, that great care should be taken, when conclusions are drawn, based on results from a single sample. However, the position of this sample in the PC plot (Fig. 1), combined with the biological and sampling variance observed for the Wadden Sea fish samples, strongly

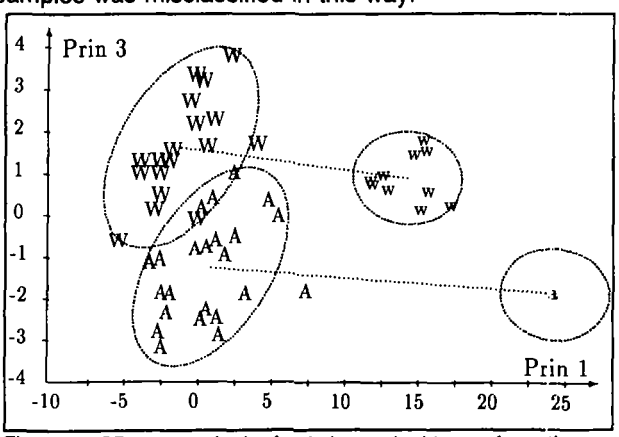

Figure 1. CB patterns in the food change by biotransformation (PC1) and seals reflect the patterns in their food (PC3). W = seal fed w-fish and A = seal given a-fish.

suggests that the CB congener pattern in Atlantic fish differs from that of the Wadden Sea fish samples. This is not surprising, as not only the sample location, but also the fish species (plaice and mackerel respectively) are different between the two groups of fish.

Difference between fish- and seal CB congener patterns. A very clear differentiation was observed between the CB congener patterns in seal- and fish samples (Fig. 1). This species difference of the CB congener patterns has been observed by other researchers[1], and it is in agreement with earlier observations[3,6]. The first PC alone is able to discriminate between the fish and seal samples by their CB congener patterns (Fig. 1), and except for one misclassification, any sample in the PC plot can be considered as belonging to a group, characterized by the species (fish, seal) and the origin of the fish given to the seal (Atlantic ocean, Wadden sea).

Component weights and CB congener degradability in harbour seal. The component weights of the CB congener in the Dutch seal samples are similar to those observed earlier in seal samples[2]. The component weights of the first versus the third PC are shown in Figure 2. The major discrimination power between the CB congener patterns in fish and in seals is the first PC, which is connected to the enzymatic metabolism of CB congeners by the harbour seals[3,6,8].

The transformation process of the CB congener patterns from fish to predator. The displacement of the fish- and seal CB congener patterns in the PC plot is composed by a metabolic contribution (mainly the first PC), and a secondary contribution (third PC) which can be ascribed to different CB congeners patterns in the fish. The CB congener patterns in fish is transformed into a harbour seal CB congener pattern (changed to the left of the PC plot), as the amount of persistent CB congeners gets higher in the predator than in the food. Since the initial position centre of the CB congener pattern in the two fish diets are vertically displaced, so are the CB congener patterns of their predator. Both groups of seals seem to transform the CB congener pattern in their diet in a similar way, as the lines, connecting centres of the predator and diet groups (Fig. 1), are nearly parallel and of the same length. There is no indication for a relatively larger impact of biotransformation due to induction of isoforms of the cytochrome P450-system[4] at higher absolute concentrations in the Wadden Sea seals compared to the Atlantic group. The differentiation between the CB congener patterns in the two groups of seals in the Dutch study can thus solely be ascribed to different CB congener patterns in their food.

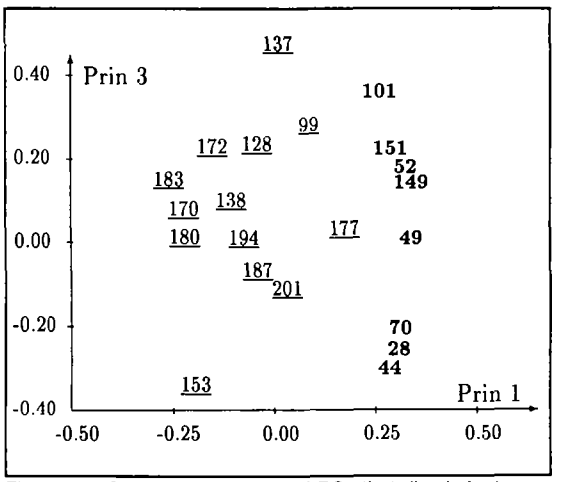

Figure 2. Component weights of PCs that discriminate between seals. CB congeners are given by IUPAC numbers (**metabolizable**, persistent).

4. Conclusions

The CB congener patterns in harbour seals, living in captivity in a Dutch study 1981-1983, and in their diet (fish), have been compared by analysis of PCs and by linear discriminant analysis. The first three PCs accounted for 80.3 % of the total variance in the seal data. The two experimental years (1981 and 1983 respectively) do not result in

different CB congener patterns within each group of samples. The CB congener patterns of the two groups of fish samples (Wadden Sea plaice and Atlantic ocean mackerel) divide in two distinct groups. Only one Atlantic fish sample is present in the data, However, the position of this sample in the PC plot, combined with the biological and sampling variance observed for the Wadden Sea fish samples, strongly indicates that the CB congener patterns in Atlantic fish and in Wadden Sea fish are different, which is a sound assumption as not only the sample location, but also the fish species are different. The CB congener patterns of the two groups of seals (given Wadden Sea fish and Atlantic ocean fish) can be separated by the discriminant analysis. A very clear differentiation between the CB congener patterns in seal samples and in fish samples is observed. The first PC is able to discriminate between the fish and seal samples. This major discrimination power between the CB congener patterns in fish and in seals, is clearly connected to the structure-related biotransformation by the cytochrome P450 system. Both groups of seals transform the CB congener pattern in their diet in a similar way. The differentiation between the CB congener patterns in the two groups of seals in the Dutch study can thus be ascribed to different CB congeners patterns in their food, whereas a possible induction of isoforms of the P450 system at the higher CB concentrations in the Wadden Sea group does not appear to have a recognizable influence. As a supplementary result, it can be concluded - based on the above results - that differences observed in CB congener patterns between separate living groups of harbour seals in Denmark is most likely due to differences in CB congener patterns of their food.

5. References.

1. Norstrom RJ, Muir DCG, Ford CA, Simon M, MacDonald CR and Béland P (1992) Indications of P450 Monooygenase Activities in Beluga (*Delphinapterus leucas*) and Narwhal (Monodon monoceros) from Patterns of PCB, PCDD and PCDF Accumulation. Mar. Environm. Res. **34**:267-272.
2. Storr-Hansen E & Spliid H (1993a) Coplanar polychlorinated biphenyl congener levels and patterns and the identification of separate populations of Harbour seals (*Phoca vitulina*) in Denmark. Arch. Environm. Contam. Toxicol. **24**:44-58.
3. Boon JP, Reijnders PJH, Dols J, Wensvoort P, Hillebrand MTJ (1987) The kinetics of individual polychlorinated biphenyl congeners in female harbour seals (*Phoca vitulina*), with evidence for structure related metabolism. Aquat. Toxicol. **10**:307-324.
4. Boon JP, van Arnhem E, Jansen S, Kannan N, Petrick G, Schulz D, Duinker JC, Reijnders PJH (1992) The Toxicokinetics of PCBs in Marine Mammals with Special Reference to Possible Interactions of Individual Congeners with Cytochrome P450-dependent Monooxygenase System: An Overview. In: Persistent Pollutants in Marine Ecosystems. Eds. C.H. Walker and D.R. Livingstone, Pergamon Press, pp.119-159.
5. Reijnders PJH (1986) Reproductive failure in harbour seals feeding on fish from polluted coastal waters. Nature **324**:456-457.
6. Boon JP, Eijgenraam F, Everaarts JM, Duinker J (1989): A Structure-activity relationship (SAR) approach towards metabolism of PCBs in marine animals from different trophic levels. Mar. Environm. Res. **27**:159-176.
7. Storr-Hansen E, Spliid H and Boon JP (1994) Patterns of chlorinated biphenyl congeners in harbour seals (*Phoca vitulina*) and in their food. Statistical Analysis. Submitted to Arch. Environm. Contam. Toxicol.
8. Boon JP, Oostingh I, Meer Jvd and Hillebrand MTJ (1994) A model for the bioaccumulation of chlorobiphenyl congeners in marine mammals. Eur. J. Pharmacol. **270**:237-251.