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The kinetics of individual polychlorinated biphenyl congeners in female harbour seals (*Phoca vitulina*), with evidence for structure–related metabolism

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Female harbour seals were held in captivity. During a period of two years, one group received contaminated fish from the Dutch Wadden Sea, while a second group was given relatively clean fish from the Atlantic Ocean. Concentrations of individual polychlorinated biphenyl (PCB) congeners were measured in fish, seal blood and occasionally in faeces of seals.

The PCB patterns within each of these three 'matrices' were highly similar, but differed between them. According to their degree of biomagnification in seal blood, PCBs could be divided into persistent congeners and congeners with lowered concentrations. This behaviour was related to molecular structural features; congeners showing lowered concentrations possessed vicinal H atoms at either a meta-para position or at an ortho-meta position. Only in the latter case the number of ortho-chlorines present influenced the toxicokinetical behaviour of the congeners; lowered concentrations were only observed for mono-ortho chlorine containing congeners.

Enzyme-mediated metabolism is the most probable cause for the relatively low contribution of such congeners to the PCB pattern in seal blood.

On a wet-weight basis, the concentrations of all congeners were lower in seal blood than in their food, but when expressed on a lipid basis, the non-metabolized congeners were biomagnified. At the end of the experiment, the PCB concentrations were significantly lower ($P < 0.001$) in the seals which had received fish from the Atlantic Ocean.

Key words: PCB; Metabolism; Marine mammal; *Phoca vitulina*

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INTRODUCTION

The harbour seal population in the Dutch part of the Wadden Sea has declined from about 3300 in 1949 to about 700 animals in 1986. The initial decline to about 1100 specimens was caused by overhunting. However, even after this was forbidden the population size continued to decrease, predominantly due to a very low reproductive rate of seals from this population (Reijnders, 1978, 1980).

It became clear that the main threat was posed by the adverse effects of the contamination of the Dutch Wadden Sea by the Rhine–Meuse estuary. Among the several organochlorines and trace metals analysed, the interest has centred on the group of polychlorinated biphenyls (PCBs) and p,p'-DDE, because of the very high concentrations of these lipophilic compounds in lipid-rich tissues of harbour seals (Reijnders, 1980). Also, PCBs caused lower reproduction rates in experiments with mink, which like the harbour seal is a fish-eating mammal and, moreover, shows a similar reproductive physiology (Jensen, 1977; Bleavins et al., 1980; Helle, 1981; Bergman et al., 1981; Reijnders, 1984).

For invertebrates and fish, equilibrium-partitioning of the parent PCB congeners (i.e. unchanged by enzymatic metabolism) between body lipids, blood and the ambient water was reported to be more important than enzymatic metabolism in PCB kinetics (Bruggeman et al., 1981, 1983; Sundström et al., 1976). In this process, the gills have an important function as sites for transfer of PCBs from blood to sea water. Marine mammals lack this pathway for elimination of apolar compounds. An alternative pathway for regulation of apolar compounds is through enzymatic biotransformation reactions that increase their water solubility, facilitating excretion via bile and urine. The inability to eliminate PCBs as parent compounds and the vital importance of metabolism have been demonstrated for terrestrial mammals by several authors (Abdel-Hamid et al., 1981; Gage and Holm, 1976; Matthews and Anderson, 1975; Matthews and Tuey, 1980; Sipes et al., 1980).

In field samples of seal organs and tissues, PCB patterns containing fewer congeners than those of gill-breathing aquatic animals originating from the same area were found (Duinker and Hillebrand, 1983b; Duinker et al., 1986). However, a clear causal relation between the PCB pattern in the source of the organochlorines, i.e. the food, and the pattern in marine mammals, is not easily obtained from correlated field data.

The induction of the activity of mixed function oxygenase (MFO) enzyme systems by PCBs (Payne, 1984), presents a possible mechanism for their chemical toxicity. MFO systems influence, among others, the rates of metabolism of (endogenous) steroid hormones, but may also be able to metabolize PCB congeners. Thus, induction of MFO systems may both increase the turnover of sexual steroid hormones and the rate of hydroxylation of certain PCB congeners, the latter depending on the chlorine substitution pattern of the individual congeners (Sundström et al., 1976). Hydroxylated and methyl-sulphone metabolites of PCBs have indeed been found in seal faeces (Jensen et al., 1975; Jensen and Jansson, 1976). Also, a considerable activity of mixed function oxygenase (MFO) enzyme systems, including aryl-hydrocarbon hydroxylase, was established even in whales from remote areas (Goksøyr et al., 1985).

In order to study the kinetics of PCBs and the effects of contaminated food on reproduction, an experiment with captive harbour seals was started at the Research Institute for Nature Management at Texel (Reijnders, 1984). One group of seals was fed contaminated fish from the Dutch Wadden Sea; the other, serving as a reference, comparatively clean fish from the Atlantic Ocean. The PCB pattern (i.e. the contribution of each individual congener to total PCB) as present in blood, was assumed to be representative for the pattern of all other organs and tissues of a seal; changes in PCB concentrations in blood on a lipid basis, were assumed to reflect the changes in the other organs in a qualitative sense.

This paper describes the pharmacokinetics; the ecotoxicological effects will be described in a separate paper.

MATERIALS AND METHODS

Origin and dosage of the food

It was impossible to get the same or even closely related fish species from the Dutch Wadden Sea and the Atlantic Ocean to serve as food for the 'treatment' and the reference groups of seals. Therefore the diet of the 'Wadden Sea' group consisted mainly of plaice (*Pleuronectes platessa*), while the diet of the 'Atlantic' group consisted mainly of mackerel (*Scomber scombrus*). The fish was frozen and stored at $-25°C$.

Both groups of seals were fed ad libitum. However, because of the higher caloric value of mackerel (e.g. 9 to 10 times higher lipid content, relatively less bones), the mean quantity of food offered to the Wadden Sea group was on average 7.5 kg fish per animal per day, while only 2.2 kg fish per seal was offered daily to the Atlantic group. The amounts of total PCB (as the sum of all 42 peaks determined) thus offered daily with the food were about 1.5 mg per seal in case of the Wadden Sea group and 0.2 to 0.3 mg for the Atlantic group (Table I). Despite the much higher lipid content in mackerel, concentrations on a wet-weight basis were still about half those of the fish from the Wadden Sea.

Composition of the groups of seals

The two groups, each consisting of 12 females, were composed of 7 seals from the Wash (U.K.) and 5 from the Museum of Natural History on Texel. The animals from the museum were born there from parents which came from the Wadden Sea. Since seals can produce their first pup at the age of 4 yr, some individuals were still sexually immature in 1981, but all animals had reached maturity in 1983.

TABLE I

Amounts of fish offered daily to the seals of the Wadden Sea (WSG) and the Atlantic group (AG) together with mean concentrations of congener 153 and Σ-PCB (as sum of the 42 peaks given in Fig. 2). In the last column, the mean daily amounts of Σ-PCB offered with the fish per individual seal, is calculated from these data.

Food	$[153]_{fish}$ ($\mu g \cdot g^{-1}$ lipid)	$[\Sigma\text{-PCB}]_{fish}$	%-lipid of WW	$[\Sigma\text{-PCB}]_{fish}$ ($\mu g \cdot g^{-1}$ WW)	Σ-PCB ($mg \cdot seal^{-1} \cdot day^{-1}$)
Wadden Sea group					
7.5 kg fish·seal^{-1}·day^{-1}					
1981 (n = 6)	1.5	11	1.9	0.20	1.5
1983 (n = 4)	2.1	14	1.4	0.19	1.5
Atlantic group					
2.2 kg fish·seal^{-1}·day^{-1}					
1981/83 (n = 3)	0.10	0.7	15	0.10	0.2–0.3

WW = wet weight.

Initially, all seals were fed mackerel. Since transition to a diet of mainly flatfish took some time, the Wadden Sea group of 1981 comprised only 6 individuals. During 1981 and 1982 the other seals completed the group, the last 2 individuals in September 1982. The composition of both seal groups is given in Table II.

Housing of the seals during the experiment

During the experiment, the two seal groups were held at the Research Institute for Nature Management at Texel in two separate basins of 30 × 6 × 1.8 m each, which were supplied with running seawater at a rate of 50 m^3 per hour. Alongside each basin, a haul-out concrete platform (30 × 3 m) was constructed.

During the experimental period (1981–1984) one seal died; the other seals did not suffer from any diseases.

Sampling for organochlorine analysis

To obtain representative samples of the fish used as food for the seals, 20 bags (containing approx. 100 fishes each) were randomly chosen. From each bag, about 30 fishes were randomly taken and were ground in a meat-grinder. The ground samples were subsampled and stored in 5 clean glass jars of 0.5 l, which were kept at −25°C until further analysis.

Blood samples for organochlorine analysis were taken from one of the rear flippers of the seals every month from October to December of 1981 and 1983. When blood samples were taken, the seals had not eaten for 20 to 30 h. About 10 ml of blood was drained from the seals into glass Vacutainer tubes (BD-3200 U, non coated); no anticoagulant was used.

TABLE II

Composition of groups of female harbour seals receiving fish from the Dutch Wadden Sea (WSG) and from the Atlantic Ocean (AG).

Seal no.	Origin	Year of birth	Remarks
WSG			
1	Wash	1973	Present in WSG in 1981 and 1983
2	Wash	1973	
3	Wash	1978	
4	Texel	1976	
5	Texel	1978	
6	Texel	1979	
7	Wash	1978	Present in 1983 only
8	Wash	1979	
9	Wash	1979	
10	Wash	1979	
11	Texel	1978	
12	Texel	1978	
AG			
11	Texel	1978	Transferred to WSG Sept. 1982
12	Texel	1978	
13	Wash	1971	Present in AG in 1981 and 1983
14	Wash	1971	
15	Wash	1972	
16	Wash	1976	
17	Wash	1977	
18	Wash	1978	
19	Wash	1979	
20	Wash	1979	Died in 1982
21	Texel	1977	Present in AG in 1981 and 1983
22	Texel	1978	
23	Texel	1978	
24	Texel	1979	
25	Texel	1979	Present in 1983 only

In the laboratory the samples were centrifuged for 15 min at 3000 rpm, which divided the sample into 3 fractions: serum (not containing any cellular material; fraction I); a fraction containing low density cellular material (thrombocytes, granulocytes and lymphocytes; fraction II) and a high density fraction, the coagulum, containing fibrin, erythrocytes and some of the lower density material previously mentioned (fraction III). On a wet-weight basis, fraction I contained 33% of the total blood sample, fraction II only 1% and fraction III 66%.

Fraction I was used completely for the determination of steroid hormones and furthermore the small amount of material in fraction II hardly contributed to the total blood sample. Therefore it was checked whether fraction III would reflect PCB concentrations in both the other fractions as well.

For routine analysis, three consecutive blood samples of each individual seal (fractions III only) were pooled to obtain enough material for capillary GC-ECD analysis of organochlorines. The period between the beginning of October and the end of December was chosen for sampling, because harbour seals have an annual sexual cycle with pups being born in July. If a seal should get pregnant during the experiment, the developing foetus would not influence PCB kinetics in the sampling period, because of the delayed implantation of the blastocyst.

Analysis of organochlorines

Since most of the procedures used have been extensively described in earlier papers (Holden and Marsden, 1969; Duinker and Hillebrand, 1978; Boon, 1985; Boon et al., 1985), only specific alterations and applications for the present study will be highlighted in this and the next sections. Organochlorines in fraction III blood samples were determined by drying about 15 ml with pretreated anhydrous sodium sulphate followed by Soxhlet extraction as described previously.

In comparison with earlier studies, a new and in some respects slightly different standard mixture was used for identification of PCB congeners with capillary GC-ECD.

The analytical methods used did not allow the detection of PCB metabolites.

RESULTS

Representativeness of fraction III for total blood samples

Fig. 1 shows the PCB patterns of the congeners detected in blood fractions I, II and III. In fraction II several peaks were below detection level, but the pattern of the compounds identified was highly similar to the virtually identical patterns of fractions I and III. The concentrations in fraction I show that substantial amounts of PCBs are associated to non-cellular material, e.g. lipoproteins. However, the absolute amounts of PCBs in fraction III (55%) > fraction I (41%) ≫ fraction II (5%).

In previous studies, virtually identical PCB patterns were observed in different parts of the same fish (Boon, 1985; Boon et al., 1984), or between different organs and tissues of mother and foetus of a harbour porpoise (Duinker and Hillebrand, 1979). From these and the present data we concluded that fraction III could indeed be used as a model representing the trends in PCB concentrations in 'total' blood.

PCB patterns

The primary objective of this study was to investigate the PCB patterns in the blood of individual seals from the two treatment groups and the pattern in their respective food. Occasionally faecal samples of single, but unknown, seals from

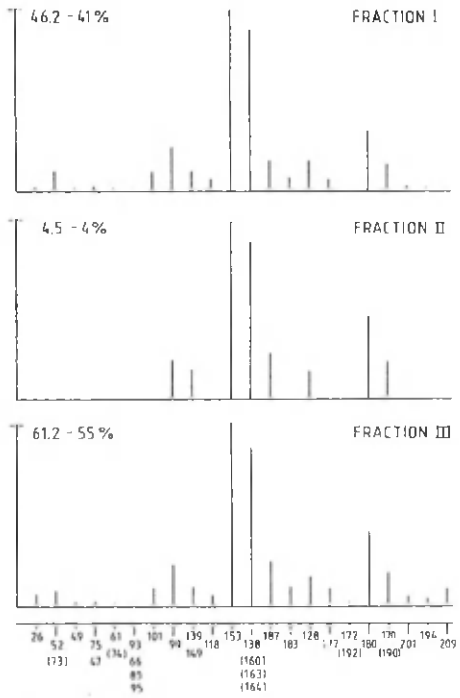


Fig. 1. PCB patterns and concentrations in the 3 blood fractions obtained after centrifugation. Numbers of PCB congeners are given in order of elution from the GC column. All concentrations are relative to height of bar. When more than one number is attributed to a single bar, the concentration is expressed on the basis of the upper congener given, but the other congeners either did not separate under our GC conditions from the first congener given, or were reported to show a difference < 0.0010 in relative retention time on the same GC column (congener number given in parentheses; Mullin et al., 1984). The numbers X/Y given in the left-hand corner of each histogram, are: X = amount of Σ-PCB (as the sum of the 22 congeners identified) in ng per fraction. Y = same amount as percentage of PCB present in the unfractionated blood sample.

each group were collected from the concrete haul-out platform in order to study the pattern of PCBs excreted in unaltered form by the animal.

The congeners reported here are a selection of those present in the standard mixture; only the congeners that were present in all samples of Wadden Sea fish ($n = 10$) are reported. This group was chosen for this purpose, because it contained the highest absolute amounts of PCBs and thus most PCB congeners could be expected to be present above detection limit.

For the study of kinetics, well separated congeners are the most important, since changes in relative peak height represent the behaviour of well defined molecular structures. The congeners 26, 49, 44, 99, 151, 118, 137, 183, 128, 177, 180, 201, 194,

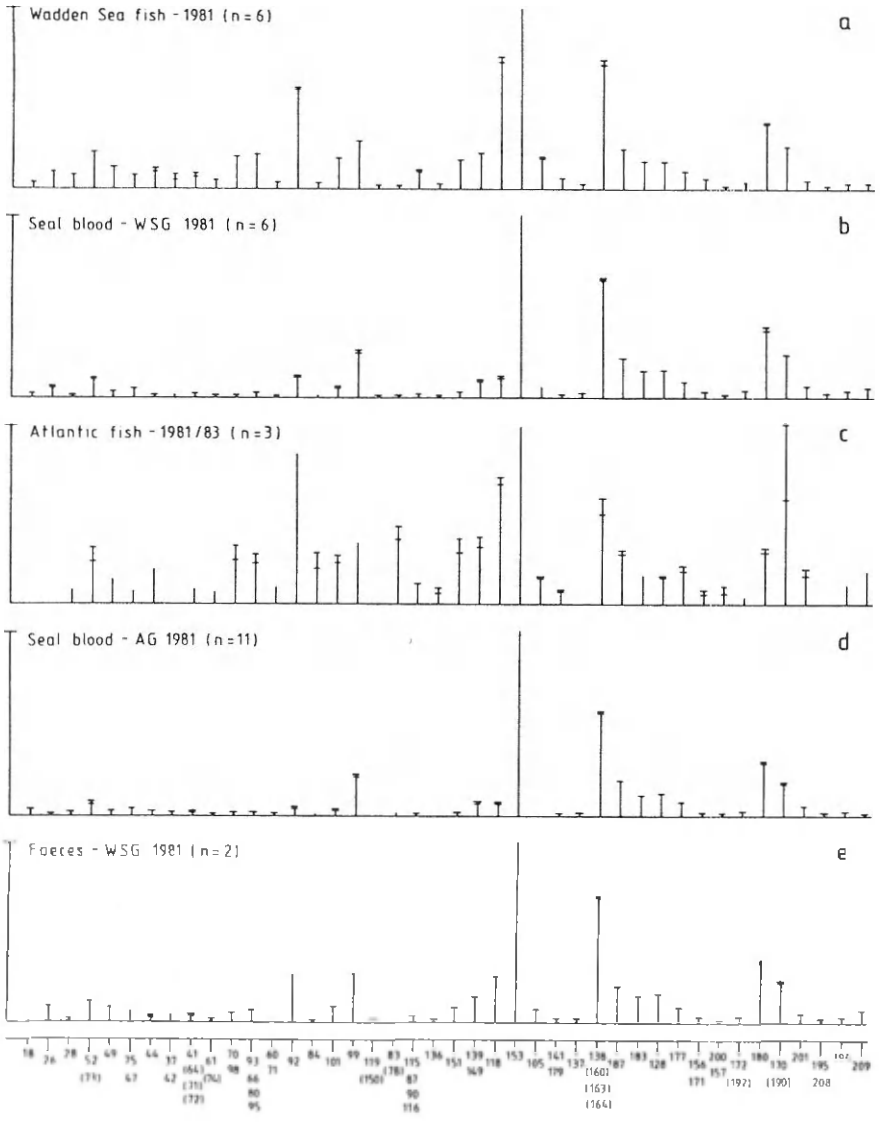
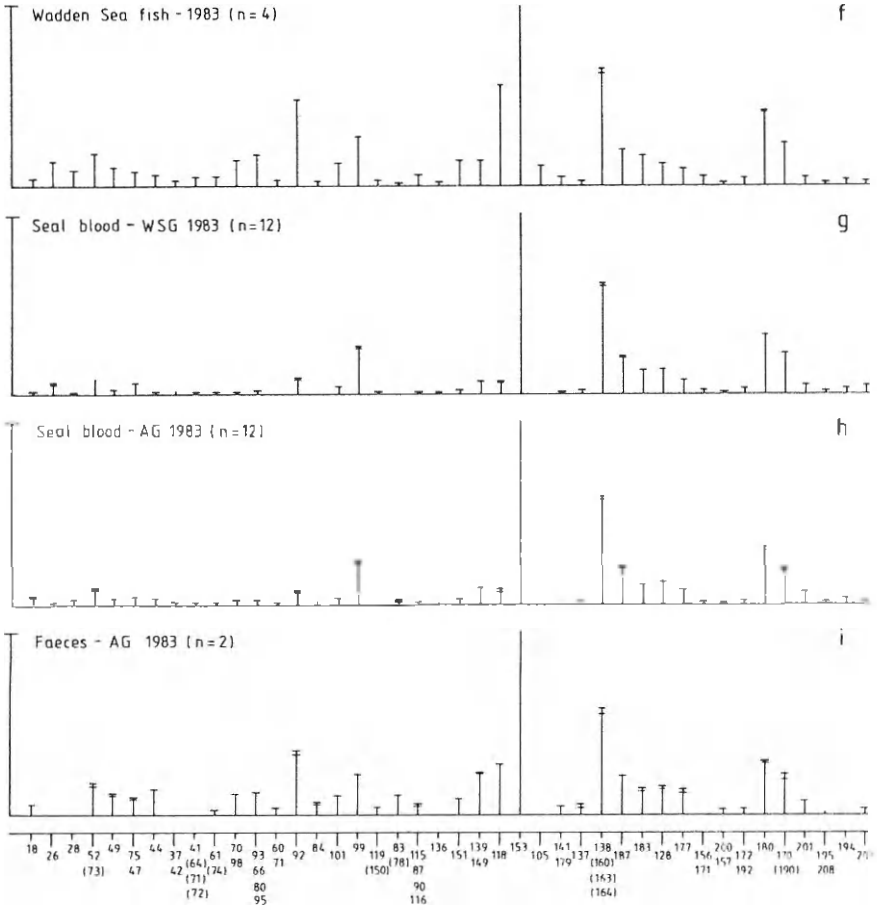


Fig. 2. PCB patterns in selected groups of fish from the Dutch Wadden Sea and the Atlantic Ocean, in blood of seals which received this fish as daily diet, and in faeces of seals. Explanation of the identification of PCB congeners is given in Fig. 1. Their mean relative concentrations are proportional to height of bar; one standard error is added between horizontal bars to the mean ratios of each congener compared to 153. However, when the SE < 1.0% of the height of 153, these bars are no longer resolved and are shown as a single bar. If no horizontal bar is given, the congener was present in only one of the n samples within a group; if no vertical bar is given, the congener was absent in a particular group (with the exception of 153, whose ratio always equalled unity).

and 209 showed unique chromatographic properties on an SE-54 capillary column (Duinker and Hillebrand, 1983; Mullin et al., 1984); therefore it is now certain that these peaks represent a single compound. Another group of congeners was shown to be separated from their possible co-eluants under our gaschromatographic conditions: 18, 28, 92, 84, 101, 136, 153, 105 and 187.

A third group of peaks could be constituted by at least two congeners which were



either present in our standard collection or were reported to differ less than 0.0010 in relative retention times by Mullin et al. (1984). Such peaks are given in Fig. 2 as co-eluting congeners, but are omitted from Table III.

The PCB patterns were made independent of concentrations by presenting the ratio of the concentration of each congener to the concentration of congener 153, which always possessed the highest concentration. For such data sets of individual samples, arithmetic means and standard errors were calculated for different groups of samples. The PCB patterns of the biotic matrices Wadden Sea fish, seal blood, and faeces were highly constant and did not depend on PCB concentrations or year of sampling, as shown by the extremely small standard errors in Fig. 2. The mean pattern in Atlantic fish closely resembled that of Wadden Sea fish. However, the standard errors were much larger than those in Wadden Sea fish, for two reasons: in these samples the peaks were very low, which gave some analytical problems for quantification because of some negative peaks occurring in the baseline; moreover, only three samples were analysed. Anyway, the PCBs in Atlantic fish caused a PCB pattern in seal blood which was virtually identical to that in seals fed with fish from the Wadden Sea. Because of the extensive similarities in PCB patterns within each biotic matrix, only a selection of the PCB patterns is given in Fig. 2. However, highly significant differences occurred between the biotic matrices fish, seal blood and faeces of seals.

If a given congener shows an equal behaviour to 153 in transfer processes from food to seal, its ratio/153 will be the same in fish and in seal blood. However, if a congener is either taken up more slowly or eliminated more rapidly than 153 by the seal, its ratio/153 will be smaller in seal blood than in food (i.e. fish). On the other hand, if a congener is either taken up faster or eliminated more slowly by the seal than is 153, its ratio/153 will be higher in seal blood. So, when for each congener its ratio/153 in seal blood is divided by its ratio/153 in food, the result shows the (net) uptake from food to seal blood of that congener relative to the behaviour of congener 153. The results of such calculations are given as values of R^* in Table III, for blood of seals fed with fish from the Wadden Sea. Due to the analytical problems, resulting in much larger standard errors, the seals fed with Atlantic fish were omitted from these calculations.

The congeners can be divided into two groups according to their kinetics in blood compared to flatfish (Table III). Group I has values of R^* between 0.12 and 0.50 (means of values for 1981 and 1983), while in group II the values of R^* are between 0.83 and 1.72. Group I contains congeners with 3–6 chlorine atoms, while group II contains congeners with 5–10 chlorine atoms. So, penta- and hexa-chlorobiphenyls were present in both groups.

PCB concentrations

Because the PCB patterns showed very high similarities within each biotic matrix, any peak or combination of peaks can be chosen to represent the trend in PCB con-

TABLE III

Relative net-uptake of PCB congeners in seal blood from food (i.e., fish originating from the Dutch Wadden Sea), expressed as values of R^*; I = Group I; II = Group II of PCB congeners. Chlorine substitution patterns of congeners that were reported to decrease the affinity to enzymatic metabolism, are marked by ● (vicinal H atoms: ● = absent; Cl substitution at both para positions: ● = present).

PCB congener	R^*				Chlorine substitution					Log P_{ow}[a]
	1981	1983	mean 1981/83		Total number of Cl atoms	Number of ortho-Cl atoms	Vicinal H atoms at ortho–meta pos.	Vicinal H atoms at meta–para pos.	Cl at para-para' pos.	
136	0.12	0.12	0.12		6	4	●			6.51
118	0.16	0.11	0.14		5	1		●	●	7.12
84	0.14	ND[b]	0.14		5	3				6.04
44	0.14	0.14	0.14		4	2				5.81
28	0.18	0.13	0.16		3	1		●	●	5.69
151	0.19	0.14	0.17	I	6	3	●			–
92	0.21	0.16	0.19		5	2	●			6.97
49	0.27	0.22	0.25		4	2				6.23
105	0.29	ND[b]	0.29		5	1		●	●	–
101	0.33	0.27	0.30		5	2	●			7.07
18	0.48	0.34	0.41		3	2				5.55
26	0.57	0.42	0.50		3	1				5.76
177	0.84	0.82	0.83		7	3	●	●	●	–
137	0.96	0.77	0.87		6	2	●	●	●	7.71
183	0.94	0.83	0.89		7	3	●	●	●	–
130	1.03	0.82	0.93		6	3	●	●	●	–
99	0.94	0.92	0.93		5	2	●	●	●	7.21
187	0.96	0.99	0.98	II	7	3	●	●	●	–
128	0.97	1.03	1.00		6	2	●	●	●	6.96
153	–	–	–		6	2	●	●	●	7.75
194	1.21	0.94	1.08		8	2	●	●	●	–
201	1.25	1.06	1.16		8	3	●	●	●	–
209	1.86	1.57	1.72		10	4	●	●	●	–

[a] Log P_{ow} (octanol–water) values are taken from Rapaport and Eisenreich (1984). [b] NDB = below detection limit in all blood samples (while the congeners selected were present in all fish samples from the Wadden Sea).

TABLE IV

Mean concentrations on a pentane-extractable lipid basis and on a wet-weight basis of congener 153 and Σ-PCB (as sum of all 42 peaks given in Fig. 2) in blood (fraction III) from seals belonging to the Wadden sea (WSG) and Atlantic groups (AG) in 1981 and 1983.

Treatment group	n	[153] ($\mu g \cdot g^{-1}$ lipid)	[Σ-PCB] ($\mu g \cdot g^{-1}$ lipid)	[153] ($\mu g \cdot g^{-1}$ wet wt)	[Σ-PCB] ($\mu g \cdot g^{-1}$ wet wt)
WSG - 81	6	6.6	27	0.0034	0.014
WSG - 83	12	6.9	25	0.0043	0.016
AG - 81	11	3.5	11	0.0023	0.0074
AG - 83	12	1.5	5.2	0.0013	0.0045

TABLE V

Significance levels of differences between the mean concentrations on a (pentane-extractable) lipid basis (LW) and on a wet-weight basis (WW) of congener-153 in blood, as given in Table IV. Statistical testing was performed with a Student's t-Test of equality of means for two groups of samples, group A being tested against group B. WSG = Wadden Sea Group; AG = Atlantic Group. NS = $P > 0.05$; * $0.01 < P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$.

Test of equality of means for two groups of samples		PCB - 153; LW	PCB - 153; WW
Group A	Group B		
WSG - 81	WSG - 83	NS	NS
AG - 81	AG - 83	**	**
WSG - 81	AG - 81	**	NS
WSG - 83	AG - 83	***	***

ntrations within that matrix. We chose the most dominant congener – i.e. 153 – nd Σ-PCB to represent the trend in seal blood (Table IV).

The statistical significance levels of differences between the mean concentrations f congener 153 are given in Table V; they were tested separately for concentrations n a pentane-extractable lipid and on a wet-weight basis. No significant differences urred between the Wadden Sea groups of 1981 and 1983, but the concentrations f congener 153 decreased significantly from 1981 to 1983 in the seals fed with tlantic fish. In 1981, the concentrations were significantly lower in the Atlantic oup than in the Wadden Sea group when expressed on a lipid basis, but not on wet-weight basis. This was due to a significantly higher lipid content in the blood the Atlantic group (mean ± SE = 0.069 ± 0.005%) compared to the Wadden a group (0.051 ± 0.005%). In 1983, the concentrations of PCBs in the Atlantic oup were reduced to only 22% of those of the Wadden Sea group on a lipid basis d, again as a result of a higher lipid content of the blood in the Atlantic group, 30% on a wet-weight basis.

A comparison of Tables I (food) and IV (blood) shows that on a wet-weight basis the concentrations of congener 153 and Σ-PCB were lower in seal blood than in fish, but that when expressed on a lipid basis, the persistent congeners (group II) were biomagnified in seal blood.

DISCUSSION

Certain structural features expected to influence rates of enzymatic metabolism are given in Table III (Sundström et al., 1976; Ballschmiter et al., 1978). Group I contains congeners that were relatively under-represented in seal blood compared to food (fish). Group II contains congeners which behaved similar to congener 153.

All congeners in group I possessed vicinal H atoms, i.e. hydrogen atoms bound to adjacent carbon atoms of an aromatic ring. The congeners in group I containing vicinal H atoms at ortho-meta positions (28, 105, 118) all possessed only one ortho-chlorine atom, while those in group II possessed either two (99, 128, 137) or three (177) ortho-chlorines. All congeners containing vicinal H-atoms at the meta-para position belonged to group I. Since these congeners contained one to four ortho-chlorines, this feature did not influence their pharmacokinetics.

If the low contributions to the PCB pattern of congeners belonging to group I had been caused by lower uptake rates, they should be enriched in the faeces of seals when compared to fish; however, the contrary was observed (Fig. 2). Moreover, laboratory experiments with fish showed that uptake rates were independent of molecular structures, while elimination rates differed largely between congeners (Bruggeman et al., 1981; Boon, 1985; Opperhuizen et al., 1986). For these reasons it is concluded that the low contribution to the PCB pattern in seals of all congeners in group I is caused by their enzymatic metabolism. Fig. 3 summarizes the minimum requirements of molecular substitution patterns for metabolism to occur. The structural features required, are the same as those reported for mice (Gage and Holm, 1976). The central role of vicinal H atoms in metabolic processes can be understood when the formation of an arene-oxide (epoxide) intermediary is assumed to be necessary for metabolism (Sundström et al., 1976; Matthews and Tuey, 1980).

Quantum mechanical calculations on PCBs resulted in an energy barrier for reaching a flat molecular structure for all ortho-substituted congeners, but not for congeners lacking ortho-chlorine substitution (McKinney et al., 1983). However, as a result of overlapping covalent bond atomic radii the energy barrier to be overcome for reaching a planar configuration will be much higher with two opposing ortho-chlorines on different rings (Fig. 3a) compared to opposing ortho-chlorine and hydrogen (Fig. 3b). Thus, PCBs can be divided into 'globular' congeners and congeners which may be able to reach a planar configuration.

Congeners containing vicinal H atoms at meta-para positions were always metabolized. Since the congeners investigated contained one to four ortho-chlorines (Table III), a planar configuration was not necessary for their metabolism and con-

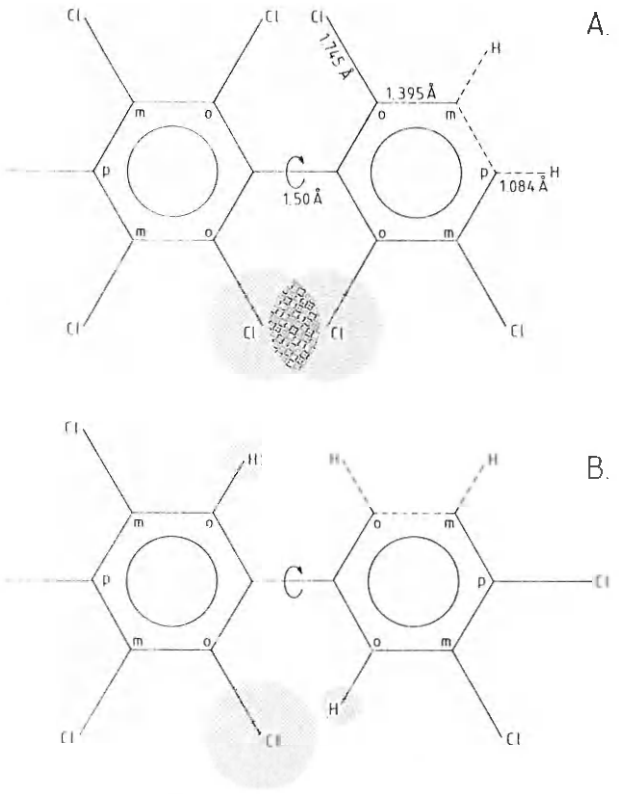


Fig. 3. Structural features of PCB congeners influencing enzymatic metabolism in female harbour seals. Areas where the principal enzymatic reaction occurs are given by dotted lines. Bond lengths are taken from McKinney et al. (1983). Single-bond covalent radii are indicated for Cl (0.99 Å) and H (0.32 Å). A. Vicinal H atoms in a meta–para position. Overlapping covalent radii for two ortho-Cl indicate that a planar configuration is highly improbable when three or four ortho-Cl are present. B. Vicinal H atoms in an ortho–meta position. One or two ortho-Cl will not oppose each other. Non-overlapping covalent radii of Cl and H show that a planar configuration is not sterically hindered in this case. However, the introduction of a second ortho-Cl opposite a vicinal H atom apparently prevented interaction with biotransformation enzymes in seals.

sequently these congeners appear to be metabolized in a 'globular' configuration.

However, when vicinal H atoms are present at ortho–meta positions, ortho-chlorine substitution appears to be of great importance; the congeners in group I (28, 105, 118) possess only one ortho-chlorine, while those in group II possess either two or three ortho-chlorines. Therefore, the ability to reach a planar configuration appears of importance for PCBs with vicinal H atoms at an ortho-meta position.

The di-ortho substituted congeners 99, 128 and 137 behaved like non-metabolizable PCBs in the present study, despite that they may also be able to reach a planar configuration without ortho-chlorines opposing each other. Instead, one ortho-chlorine will oppose the vicinal H atom at the ortho-position of the other ring. Steric hindrance by this ortho-chlorine atom may have prevented enzymatic interaction of these congeners in seals.

Compounds interacting with cytochromes *P*-448* are generally flat and are often attacked next to a bay region, while those interacting with cytochromes *P*-450* are globular and are attacked at other positions (Parke et al., 1985). Planar PCBs also form a bay region. Therefore, the apparent necessity of PCB congeners with ortho–meta vicinal H atoms to reach a planar configuration for metabolism, indicates that cytochrome *P*-448 may be involved. In contrast, cytochrome(s) *P*-450 may be involved in the metabolism of PCBs with vicinal H atoms at a meta–para position.

Their ability to reach both a globular (rings perpendicular) as well as a planar configuration may also explain the nature of mixed type MFO-induction by mono-ortho chlorine containing congeners. Congeners 105 and 118 are known to be mixed-type MFO-enzyme inducers in rats (i.e. the synthesis of both cytochromes *P*-448 and *P*-450 is induced), while congener 77, which only lacks the ortho-chlorine, is a pure cytochrome *P*-448 inducer (Safe et al., 1982).

The effect of metabolism on the toxicity of PCB-congeners is still uncertain, since the metabolites formed can either be rapidly excreted (detoxification), or the stable metabolites and/or highly reactive intermediates in their formation route – such as arene oxides – may react with autochtonous molecules (intoxication). A detoxifying property of PCBs metabolized in a planar configuration is that the bay region (often preventing epoxide-hydrolase in PAHs) is lost upon the termination of the enzymatic interaction because the molecule is then again free to rotate along its inter-ring bond.

A high constancy in PCB patterns within biotic matrices as observed in the present study was also observed for other marine species (Duinker et al., 1983a; Boon et al., 1985). However, between species PCB patterns may differ, hampering comparisons of PCB concentrations on the basis of total PCB. The pattern observed in seal blood in the present study was very similar to that of organs and blubber of a harbour porpoise (Duinker and Hillebrand, 1983) and seals (Duinker et al., 1986) obtained from field samples. In general, the PCB pattern of these coastal marine mammals differed more from those of gill-breathing aquatic species, than the latter differed from each other. This is consistent with the hypothesis that differences between animal species in metabolic capacity play a major role in the long term establishment of PCB patterns in the marine biota. With regard to the kinetics of

*Cytochromes *P*-448 and *P*-450 = 3-methylcholanthrene- and phenobarbital-inducible isozyme types of cytochrome *P*-450; respectively.

PCB congeners it is generally accepted that equilibrium-partitioning of the parent compounds between body lipids, blood and the ambient sea water is more important than excretion after enzymatic metabolism in gill-breathing animals. Only the first type of behaviour can be modelled adequately relating the degree of bioaccumulation to values of log *P* octanol–water. However, some metabolic capacity for PCB congeners up to the pentachlorobiphenyls has been demonstrated in fish (Bruggeman, 1983) and a polychaete species (Goerke and Ernst, 1986).

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Acute toxicity of three detergents and two insecticides in the lugworm, *Arenicola marina* (L.): a histological and a scanning electron microscopic study

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The toxicity of three detergents (sodium dodecyl benzene sulfonate, sodium dodecyl sulfate and Triton X-100) and two insecticides (Carbaryl and Parathion-ethyl) on the lugworm, *Arenicola marina*, was investigated. The 48-h LC_{50} values were established and the morphological alterations in epidermis, gills and intestine were analysed by light and scanning electron microscopy. The three detergents were equally toxic (LC_{50} from 12 to 15 mg · l^{-1}) while the insecticides were more potent (LC_{50} = 7.2 and 2.7 mg · l^{-1}, for the Carbaryl and the Parathion-ethyl, respectively). The gills and the epidermic receptors were the most sensitive sites of the lugworm, while the thoracic epidermis was the most resistant of the structures studied.

Key words: Toxicity; Detergent; Insecticide; Lugworm; Morphological damage

INTRODUCTION

Detergents and insecticides deriving from industrial, agricultural and domestic use are among the most important pollutants of the coastal environment. The chemicals are carried in the drainage water from large- and small-scale farming activities through outlets and rivers to estuaries where they may enter food chains and concentrate in coastal seawater and sediments (Walsh, 1972; Alzieu and Maggi, 1974; Bellan, 1976; Young et al., 1977; Siron and Giusti, 1985).

Due to the growing appreciation of the importance of the coastal environment and to the increased use of these chemicals, estuarine and marine coastal animals have been subjected to toxicological studies. Acute toxicity of non-ionic and ionic

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