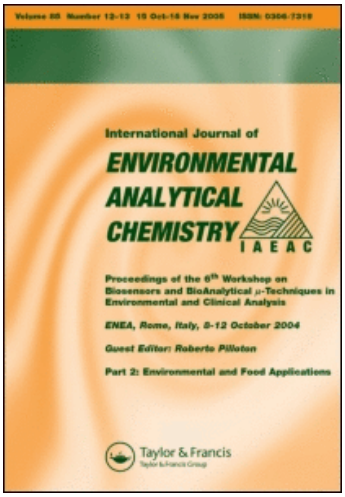

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Review: Biological Activity, Determination and Occurrence of Planar, Mono- and Di-Ortho PCBs

Pim De Voogt[a]; David E. Wells[b]; Lars Reutergårdh[c]; Udo A. Th. Brinkman[d]
[a] Institute for Environmental Studies, Free University, Amsterdam, The Netherlands [b] DAFS, Marine Laboratory, Aberdeen, Scotland [c] Swedish Environmental Protection Agency, Solna, Sweden [d] Department of Analytical Chemistry, Free University, Amsterdam, The Netherlands

To cite this Article De Voogt, Pim , Wells, David E. , Reutergårdh, Lars and Brinkman, Udo A. Th.(1990) 'Review: Biological Activity, Determination and Occurrence of Planar, Mono- and Di-Ortho PCBs', International Journal of Environmental Analytical Chemistry, 40: 1, 1 — 46
To link to this Article: DOI: 10.1080/03067319008030516
URL: http://dx.doi.org/10.1080/03067319008030516

Intern. J. Environ. Anal. Chem., Vol. 40, pp. 1–46
Reprints available directly from the publisher
Photocopying permitted by license only

Review

BIOLOGICAL ACTIVITY, DETERMINATION AND OCCURRENCE OF PLANAR, MONO- AND DI-ORTHO PCBs

PIM DE VOOGT
Institute for Environmental Studies, Free University, P.O. Box 7161, Amsterdam, The Netherlands

DAVID E. WELLS
DAFS, Marine Laboratory, Victoria Road, Aberdeen, Scotland

LARS REUTERGÅRDH
Swedish Environmental Protection Agency, S-17185, Solna, Sweden

UDO A. TH. BRINKMAN
Department of Analytical Chemistry, Free University, Amsterdam, The Netherlands

(*Received 21 March 1990*)

Some of the 209 polychlorinated biphenyls (PCBs) are stereochemically similar to the planar 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and, because of this similarity, exert biochemical activity and toxicity comparable to that of TCDD. The molecular dimensions and the planarity of the PCB molecule appear to be important for the interaction with the TCDD receptor. These structural requirements, in turn depend on the number of ortho chlorine atoms and the presence of two para and at least two meta chlorine atoms on the biphenyl skeleton. Planar PCBs (i.e., without ortho chlorines), their mono- and some of their di-ortho substituted congeners have inductive properties similar to 3-methylcholanthrene- or mixed-type induction of mixed-function oxidases. These congeners also appear to have the greatest toxic potential.

Planar PCBs and some of their mono- and di-ortho substituted congeners occur in very low concentrations in commercial PCB mixtures. Hence, their environmental occurrence is relatively low compared to that of abundant PCB congeners. The gas chromatographic (GC) determination of planar PCBs therefore requires relatively large sample sizes and/or the use of retention gaps or injection systems which allow the introduction of relatively large sample volumes, to obtain sufficiently low limits of detection. The isolation and identification of single PCB congeners in general, and that of less abundant ones in particular, still is rather difficult and often imprecise and/or inaccurate—even with the currently available high-resolution capillary GC columns—because of the complex chromatograms. Specific sample pretreatment techniques, using carbon chromatography or pyrenyl-coated silica columns for group separation, and/or multi-dimensional GC separations are available nowadays for the proper determination of planar PCBs.

From the limited amount of data available for individual PCBs, it appears that the environmental concentrations of the biochemically active PCBs usually are several orders of magnitude higher than those of TCDD. Hence, their environmental significance in terms of toxic potential—especially that of

PCBs 105, 126 and, possibly, 118 and 156—is likely to be even greater than that of TCDD. Although some experimental evidence exists with regard to the causal link between PCB contamination and reproductive failure in fish-eating mammals, the amount of data on individual PCBs is insufficient to indicate PCB toxicity as the cause of local extinction of mammals such as, e.g. the otter and the porpoise, in northwestern Europe. The very possibility of this causal link, however, calls for immediate global monitoring action for planar and mono- and di-ortho substituted PCBs.

1. INTRODUCTION*

Polychlorinated biphenyls (PCBs) have the empirical formula $C_{12}H_{10-n}Cl_n$ ($n=1$–10). There are 209 chlorobiphenyl congeners which can be divided into nine isomeric groups and decachlorobiphenyl (Table 1), each of which has a systematic number.[1]

Commercially, PCBs are manufactured by the batch chlorination of biphenyl which results in technical mixtures containing a given chlorine content, depending on the duration of the chlorination process.[2] Although all 209 of the PCB congeners have been synthesised,[3] the reaction conditions in the commercial process tend to favour specific substitution reactions leading to particular compositions of the chlorinated products. To date some 20 congeners are thought to be absent from all of the technical mixtures.[4] The most abundant congeners in the various technical mixtures are given in Table 2.

The use and application of the technical mixtures[2] has eventually led to significant levels of the more common PCBs being present in almost every compartment of the environment.[12–14] Concern has increased over the years with regard to the ecotoxicological and human health implications of the environmental presence of materials containing PCBs.

Table 1 Nomenclature, number of isomers, molecular weights and chlorine content of isomeric groups of PCBs

Structural formula	*Name (-chloro biphenyl)*	*Number of isomers*	*IUPAC systematic numbering*	*Mol. wt.*	*% Cl*	*Number of isomers identified in commercial mixtures*
$C_{12}H_9Cl$	mono	3	1–3	188.65	18.79	3
$C_{12}H_8Cl_2$	di	12	4–15	233.10	31.77	12
$C_{12}H_7Cl_3$	tri	24	16–39	257.54	41.30	23
$C_{12}H_6Cl_4$	tetra	42	40–81	291.99	48.65	41
$C_{12}H_5Cl_5$	penta	46	82–127	326.43	54.30	39
$C_{12}H_4Cl_6$	hexa	42	128–169	360.88	58.93	31
$C_{12}H_3Cl_7$	hepta	24	170–193	395.32	62.77	18
$C_{12}H_2Cl_8$	octa	12	194–205	429.77	65.98	11
$C_{12}HCl_9$	nona	3	206–208	464.21	68.73	3
$C_{12}Cl_{10}$	deca	1	209	498.66	71.10	1

*A list of abbreviations is given at the end of the paper.

Table 2 Major congener composition of technical PCB mixtures (in wt. %)*,[a]

PCB no.	*Type*[b]: *Origin*: *% Cl*:	*A30 FRG 42*	*A40 FRG 48*	*A50 FRG 54*	*A60 FRG 64*	*1221 USA 21*	*1242 USA 40–42*	*1254 USA 52–54*	*1260 USA 60*	*D103 CSSR 48*	*Kan. Japan ca. 45*
1						32.1					
2						2.7					
3						19.1					
4						4.8	4.0			2.4	
5+8		6.1				10.2	9.0			7.1	
6						3.1					
18		9.9	3.8				9.4			8.5	3.5
15		9.0				3.6					
17							2.9			5.2	
16							3.2			5.8	
32							2.2				
26		2.1									
31		6.8	2.4				4.5			9.1	5.2
28		9.9	4.0				13.3			13.0	
20							3.6				
21+33		4.6					2.8			7.0	
22							2.6				
52		3.1	7.3	6.8	5.0		4.1			4.4	2.8
75							2.2				
49			4.1				3.3				
44		3.0	6.6	3.3							2.0
42								2.2			2.1
41			3.5								2.0
35										3.6	
39										2.3	
37		3.2								2.5	
61		2.2									
91								5.0	3.2		
121								3.5			
74							2.0				
70								4.8			3.1
80		2.5									
66		2.3	5.7					2.2			2.2
60			3.1								
95				2.3	3.9						3.1
84				2.7							
101			2.3	6.1	4.1			7.0	5.0		3.3
99				2.5				6.1			
97								2.6			
87+90+116				3.5				3.8			
110			2.8	9.7	3.6			8.5	3.6		
151					4.7						
149				4.1	9.6			3.6	9.5		3.3
118		2.5	6.7	10.5	1.0			8.1	2.0		2.8
153				3.2	8.6			3.3	8.2		4.6
132				3.1	4.6			2.0	2.8		
138				6.0	11.3			4.2	5.0		4.9
187					3.8						
183					3.1				2.6		
167					4.9						

Table 2 (*continued*)

PCB no.	*Type*[b]: *Origin*: *% Cl*:	*A30 FRG 42*	*A40 FRG 48*	*A50 FRG 54*	*A60 FRG 64*	*1221 USA 21*	*1242 USA 40–42*	*1254 USA 52–54*	*1260 USA 60*	*D103 CSSR 48*	*Kan. Japan ca. 45*
174					3.4						
185									5.6		
181									2.7		
171									4.3		
180					8.9				7.2		3.9
170					5.2						
193									2.3		
194									2.2		

[a]Sources: Albro *et al.*;[5–7] Duinker and Hillebrand;[8] Lišhák;[9] Kannan *et al.*;[10] Schneider *et al.*[11]
[a]Only percentages >2 given.
[b]Clophens: A30–60, Aroclors: 1221–1260, Delor; D103, Kanechlors: mixture of Kan 300, 400, 500 and 600 (1:1:1:1, v/v).

The PCB problem was recognised over 20 years ago; this resulted in extensive reports and reviews on environmental contamination levels being written.[15–17] However, only a small proportion of these studies discussed the distribution and occurrence of individual PCBs as part of the analysis of environmental samples. Although the approach to PCB determination has rapidly changed in recent years, it is still easy to understand the historical reasons for the inertia to measure individual congeners. Total PCB levels are often required for legislative monitoring programs, and for the measurement of environmental effects caused by the PCB formulations as such. Early analytical techniques using packed column gas chromatography could only measure a "total PCB" content by summing the few—seemingly, fully—resolved peaks, or by conversion of all PCBs into a single compound.[18,19] In particular perchlorination has been a successful technique,[19] but it should be considered as obsolete now because it often turned out to give an overestimation of the total PCB content. Besides, pattern recognition was made impossible by the technique. Only with the development of high-resolution capillary gas chromatography (GC) with electron capture detection (ECD) and/or mass spectrometry (MS) has it become possible to determine more accurately the presence of individual PCBs on a routine basis.

The toxicity of the PCB formulations to wildlife has been known and studied for the past 20 years.[14,20,21] It has also been known for some time that specific congeners, such as, e.g., 3,4,3′,4′-tetraCB (PCB 77) and 3,4,5,3′,4′-pentaCB (PCB 126), are more toxic than most other congeners. Recently, a series of systematic studies has been reported,[22–24] in which the most toxic PCB congeners have been assessed on the basis of enzyme induction and receptor binding affinity. The PCBs which are substituted in both para positions and at least two meta positions, i.e., congeners 77, 126 and 169 (3,4,5,3′,4′,5′-hexaCB) are the most biochemically active as well as the most toxic ones. Congener 81 (3,4,5,4′-tetraCB) also fulfils the structural requirements given above, but appears to be somewhat less toxic. These congeners belong to the class of so-called planar or coplanar PCBs, i.e., the

Table 3 Systematic numbering of planar, mono-ortho and di-ortho PCBs[a]

No.	*Type*	*Structure*	*No.*	*Type*	*Structure*
Tetrachlorobiphenyls			*Hexachlorobiphenyls*		
77	P	3,3',4,4'	157	M	2,3,3',4,4',5'
81	P	3,4,4',5	158	D	2,3,3',4,4',6
			166	D	2,3,4,4',5,6
			167	M	2,3',4,4',5,5'
			168	D	2,3',4,4',5',6
Pentachlorobiphenyls			169	P	3,3',4,4',5,5'
105	M	2,3,3',4,4'			
114	M	2,3,4,4',5			
118	M	2,3',4,4',5	*Heptachlorobiphenyls*		
123	M	2',3,4,4',5	170	D	2,2',3,3',4,4',5
126	P	3,3',4,4',5	180	D	2,2',3,4,4',5,5'
			189	M	2,3,3',4,4',5,5'
			190	D	2,3,3',4,4',5,6
Hexachlorobiphenyls			191	D	2,3,3',4,4',5',6
128	D	2,2',3,3',4,4'			
137	D	2,2',3,4,4',5			
138	D	2,2',3,4,4',5'	*Octachlorobiphenyls*		
153	D	2,2',4,4',5,5'	194	D	2,2',3,3',4,4',5,5'
156	M	2,3,3',4,4',5	205	D	2,3,3',4,4',5,5',6

[a]P = planar PCB, M = mono-ortho congener, D = di-ortho congener.

laterally substituted PCBs which do not possess ortho chlorine substituents which would restrict free rotation around the central phenyl–phenyl bond. Introduction of one or two chlorine atoms ortho to this central bond diminishes, but does not necessarily eliminate certain biochemical activities inherent in planar compounds. For all eight mono-ortho substituted PCBs and for at least five of the thirteen di-ortho substituted PCBs the presence of biochemical activities comparable to those of the planar congeners have been demonstrated.[24]

The present paper discusses the four planar congeners and the mono-ortho and di-ortho substituted PCBs. Their structures are given in Table 3. It is our aim to briefly review the current knowledge about the biological activity of individual PCBs, to evaluate existing methods for the determination of planar, mono- and di-ortho PCBs and to review environmental levels of the planar congeners, with emphasis on northwestern Europe. An attempt will be made to relate these findings qualitatively to the structure, i.e., the chlorine substitution pattern and chlorine position, of the various PCBs and to recommend future investigational efforts in this particular field.

2. BIOLOGICAL ACTIVITY

2.1 Activity

The toxicity of PCBs has been known for some considerable time. Although a

small number of PCB congeners were known to be extremely toxic, viz. PCBs 77, 126 and 169, it was initially thought that the concentrations of these particular compounds in the environment were too low to have a real impact. Therefore, toxic impurities such as the PCDDs and PCDFs and, especially, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) often present at trace levels in the industrial PCB formulations were investigated. However, more recently there have been reports[25-28] indicating that the intrinsic "PCB" toxicity may be due to the presence of planar molecules and that the toxic effects of TCDD, TCDF and planar PCBs should be added because they all bind to a common cytosolic aryl hydrocarbon receptor.

The more common biological effects which have been observed in the laboratory, mainly with terrestrial animals, include hepatic damage, dermal disorders, reproductive toxicity, thymic atrophy, body weight loss, immunotoxicity and teratogenicity.[17,29] There have been extensive reports, particularly dealing with the Baltic area, of similar distressing effects on seals (*Halichoertus grypus* and *Pusa hispida*[30]). The accounts of the chronic toxicological effects and physiological disorders in the seal populations have firmly implicated the elevated levels of PCBs as one of the probable causes. In the Dutch Wadden Sea the decline of harbour seals, *Phoca vitulina*, is thought to be related to high PCB concentrations in the diet.[31] A group of test seals fed fish from the Wadden Sea exhibited extensive reproductive failure in contrast to a similar group fed with fish from the North Atlantic.[32,33] There was a significant decrease in the plasma retinol (the major intermediate in transport of vitamin A) and thyroid hormone levels in blood, taken from the seals fed with the Wadden Sea fish; this is a mechanism similar to the causal effect observed in rodents as a result of exposure to planar PCBs.[34] Until recently, when reviewing the literature on the concentration of PCBs in seals, the correlation between the experimental values and the physiological effects was obscured by the high variance in the PCB levels.[35] The findings of Brouwer *et al.*,[34] however, demonstrate the presence of a causal mechanism in seals, and suggest a similar causality in other endangered species.

Although the relevance of any chemical attack on a community is greatest at the ecosystem level, the causal relationships are best understood at the sub-cellular level. The progress in the analytical abilities to separate and identify specific toxicants at the ultra-trace level ($pg \cdot g^{-1}$) and to relate these data to known sub-cellular changes, e.g., enzyme induction, may be a significant step forward in confirming any such causal link.

In animals exposed to PCBs the physiological effects are preceded by the induction of several enzymes including the hepatic and extrahepatic drug metabolising enzymes. Xenobiotics are known to induce liver microsomal enzyme activities, e.g. in mammals and fish. Lipophilic xenobiotics are eliminated in the liver by mixed-function oxidase (MFO) systems, which convert these to more water-soluble metabolites.[36] The MFO activities of xenobiotics can be characterised by several types of inductive properties, two of which are of primary interest for PCBs and related compounds: (a) the phenobarbital (PB) and (b) the 3-methylcholanthrene (MC) type inducing properties. Some fifty of the PCB congeners are known or suspected to be inducers,[36] but the majority, in particular

the lower chlorinated congeners, is inactive or ineffective, most likely as a result of rapid metabolism.

PB-type compounds induce several cytochrome P-450 (b, e and a) isoenzymes, which stimulate the metabolism of a large variety of compounds. Increased levels of a cytosolic receptor protein occur when PB-type inducers are administered. MC-type inducers enhance metabolism of a more limited group of substrates, induce the formation of cytochrome P-450 (c, d and a), and induce aryl hydrocarbon hydroxylase (AHH) and ethoxy resorufin-O-deethylase (EROD). Some PCBs exhibit a mixed PB/MC-type inducer activity.

The PCBs most active in elevating the cytosolic receptor levels exhibit the lowest affinity for the receptor protein and do not induce EROD activity; in contrast the most toxic PCBs, which are approximately stereoisomeric with TCDD, both induce EROD and AHH and bind with high affinity to the receptor, but do not increase the cytosolic receptor protein level.[37] A high correlation has been found between affinity to the receptor and AHH/EROD induction, and between induction and toxicity (weight loss, thymic atrophy).[24,38] However, McKinney *et al.*[39] stated that at least two different receptor types may be present, which would imply that the relationship between toxicity and induction may not be valid.

Toxicity and induction are both considered to involve an initial binding of the congener to the same (arylhydrocarbon, Ah,[40]) receptor.[38] TCDD is known to bind to the Ah receptor which subsequently translocates to the cell nucleus. The TCDD-receptor complex increases the production of mRNA responsible for synthesis of all proteins controlled by the Ah locus. One of the common structural features between TCDD, TCDF and some of the PCBs is the ability of PCBs to adopt a planar configuration, such as that of TCDD, due to the unsubstituted ortho positions on the biphenyl molecule. Consequently, planar PCBs are thought to act in a manner similar to TCDD, interacting with the Ah locus, while the non-planar PCBs are unable to do so.

2.2 Structure-Activity Relationships

The ability for a PCB to adopt a planar configuration depends on the position of the chlorine substituents. The preferred conformation for all PCBs, including the non-ortho substituted congeners, is a non-planar one.[41] With no ortho chlorine substitution, there is a minimum steric hindrance of rotation about the phenyl–phenyl bond and the planar configuration can be adopted with a minimum energy requirement. The introduction of a chlorine atom ortho to this bond significantly increases the energy requirement for free rotation about this bond, which is enhanced by additional substitution in the ortho positions. Such changes in substitution pattern have a marked effect on the activity of the PCB congener. The position of the chlorine atom has a greater influence than the number of substituents, particularly for between three and seven chlorine atoms. The PCBs which induce microsomal AHH are substituted in both para positions and at least two meta positions. (Even for the 4,4'-dichlorobiphenyl (PCB 15), however, some 3-MC type inducing activity has been reported[42]). The addition of one or more

Table 4 Classification of PCB congeners based on cytochrome P-450 inductive properties[a]

Group[b]	*Structural characteristics*	*Type of induction*
I A (77, 126, 169)	Non-ortho substituted	3-Methylcholanthrene
I B (15, 37, 81)	Non-ortho substituted	Mixed or phenobarbital
II	Mono-ortho substituted	Mixed
III	Di-ortho substituted; one or two ortho-Cl adjacent to a meta-Cl, or both ortho in same ring	Mixed
IV	Di-, tri- and tetra-ortho substituted, with no ortho-Cl adjacent to a meta-Cl	Phenobarbital

[a]Sources: Parkinson *et al.*;[22] Safe *et al.*[23].
[b]PCB IUPAC numbers given for members of groups IA and IB.

ortho substituents diminishes, but does not necessarily eliminate, the AHH-inducing activity.

The three PCBs which have the para/meta substitution only and are biochemically the most active ones (77, 126 and 169), are only present in trace quantities in commercial mixtures, but, in conjunction with traces of PCDFs and PCDDs, may be responsible for the toxicity of these formulations.[43] PCB 81 also meets the requirement of para/meta substitution only, but its activity is somewhat less. The mono-ortho substituted PCBs (105, 114, 118, 123, 156, 157, 167 and 189) induce AHH and resemble the mixed-type inducers. This mono-ortho substitution does not eliminate the binding affinity.[24] Amongst the di-ortho substituted PCBs at least five (128, 138, 158, 166 and 170) have a mixed-type induction activity.[24]

Parkinson *et al.*[22] and Safe *et al.*[23] distinguished four categories of congeners based on the cytochrome P-450 inductive properties (cf. Table 4). This classification appears to hold not only for inductions, but also for toxicity, monooxygenase induction and receptor binding affinity.[24,38]

Sassa *et al.*[44] divided the PCBs into four groups with different conformation. Active PCBs have relatively flexible conformations, reflected in greater probability of planarity, whereas inactive ones have rigidly angulated conformations. This division was based on the calculation of conformational energies as a function of the torsional angle between the phenyl rings. The method has been criticised, however, since the authors found that a torsional angle of 90° was the minimum energy structure for all PCBs, which is not correct. McKinney *et al.*[45] used a similar classification using apparent minimum energy conformation angles and discerned four categories with torsional angles of 0, 42, 70 and 90°, respectively. In further work, McKinney *et al.*[46,47] arranged the PCBs on a more continuous scale based on the relation between binding affinities and equilibrium constants derived from data on polarisability and the distance between receptor and effector. The ortho-effect was an important parameter in determining the distance, and the

dispersion energy gain from a planar alignment may assist in overcoming the small rotational barrier to planarity in non-ortho substituted PCBs.

McKinney *et al.*[46,47] proposed a model for the interaction with the Ah receptor. The essential molecular parameters in the model are the polarisability of the congener and the distance separating the receptor and the congener. Polarisability may be the determining factor in interactions between two molecules when permanent dipoles are weak or absent and/or similar (i.e. in a group of compounds having high chemical/structural similarity), and where ionisation energies are nearly identical for all compounds. This is so because dispersion interactions depend on the square of the polarisability. Since polarisability is roughly proportional to the number of electrons, for large molecules the dispersion interactions can be expected to be dominant. Thus, in a group of molecules with similar shapes and sizes, and when the receptor is assumed to be equally accessible, polarisability would be the main factor determining binding affinity (together with the receptor–molecule distance).

In their model, McKinney *et al.* developed an expression for the binding equilibrium constants, assuming that receptor and molecule would be oriented parallel to each other. They used binding affinities of Bandiera *et al.*,[48] who determined competitive binding of 14 PCB congeners to the cytosolic TCDD receptor. PCB EC_{50} concentrations were defined as the concentration necessary to reduce the specific binding of [^{3}H]TCDD to 50% of the maximal value in the absence of a competitor molecule.

Safe *et al.*[23] found a good correlation between the same binding affinities and steric, hydrophobic, electronic and hydrogen bonding factors. They also demonstrated an excellent correlation (see Figure 1) of the binding affinities with AHH induction potencies (cf. previous section). Clarke[36] used principal components analysis to predict the type of inducing activity of individual congeners. The principal components were extracted from variables coded for the chlorine positions on the biphenyl molecule. Five groups of congeners dominated, viz. MC inducers, mixed-type inducers, PB-type inducers, weak PB-type inducers and suspected PB-type inducers. This type of analysis can, in principle, be used for the prediction of activities for hitherto non-tested congeners.

The findings on the activities of individual PCB congeners and the established structure-function relationships are summarised in Table 5.

2.3 Toxic Equivalency

Poland and Glover[49] first identified a high-affinity cytosolic receptor protein and its part in stereoselective AHH activity. Bradlaw and Casterline[50] identified this induced AHH enzyme activity as a possible screening technique to detect toxic, planar polychlorinated organic compounds. Rat hepatoma H-4-IIE cell cultures were used to produce an ED_{50} response (the estimated dose needed to produce 50% of maximum enzyme induction) in a wide range of matrices including fish extracts containing planar chlorobiphenyls. Safe[21] applied this *in vitro* AHH/EROD-induced bioassay to an *in vivo* animal model using the Wistar rat. There was a high correlation between loss in body weight ($r=0.93$), thymic atrophy

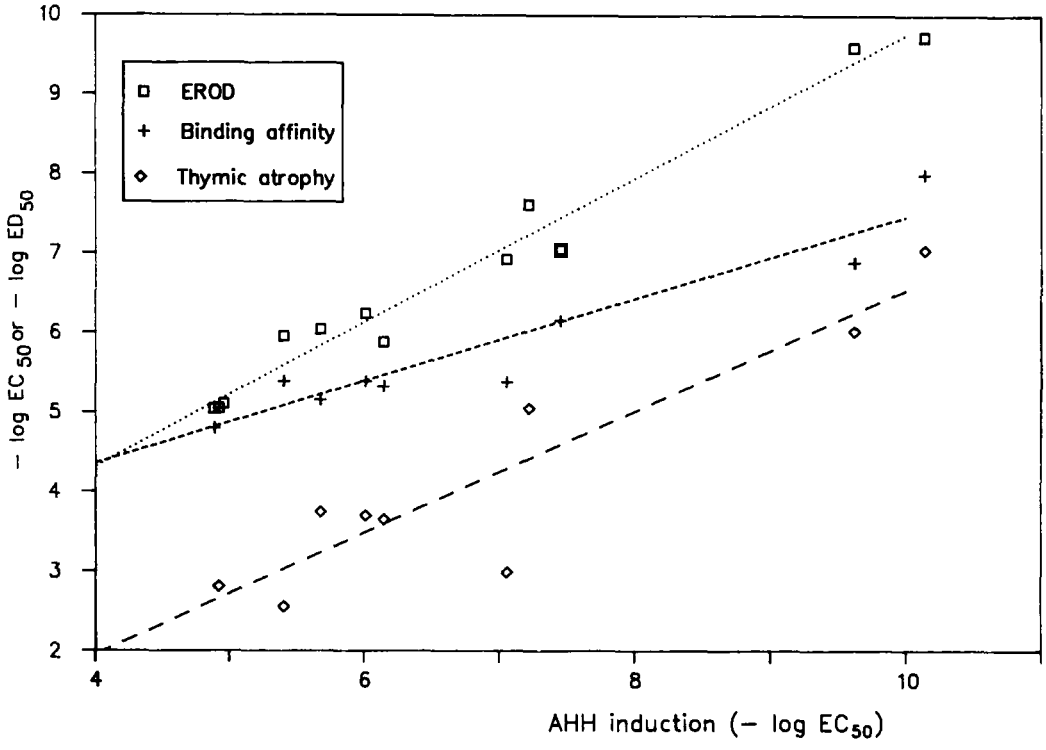

Figure 1 Plots of the *in vitro* AHH induction (expressed as $-\log EC_{50}$) vs. *in vitro* EROD induction ($-\log EC_{50}$), receptor binding affinity ($-\log EC_{50}$) and *in vivo* rat toxicity (thymic atrophy, $-\log ED_{50}$) for planar and mono-ortho PCBs and TCDD (cf. Table 5). Adapted from Goldstein and Safe.[38]

($r=0.92$) and the *in vitro* enzyme induction (cf. Figure 1). These studies with some fifteen PCDFs, five PCDDs and eight PCBs have formed the basis of the current approach in determining the toxicity of these AHH inducers in terms of that of the most toxic compound, TCDD.

The normalisation based on the TCDD Toxic Equivalence Factor (TEF) has permitted a new perspective in assessing the inherent toxicity of this series of planar molecules, their concentration in a particular species and their likely biological impact. Tanabe *et al.*,[25] Kannan *et al.*[27] and Tanabe[14] have developed this by expressing the concentration of these planar molecules in terms of the TCDD equivalent concentration, which is a simple multiple of the concentration of the planar molecule determined in the species (in $pmol \cdot g^{-1}$ or $pg \cdot g^{-1}$) and the TEF determined by Sawyer and Safe[51] and Safe.[52].

The *in vitro* EC_{50} AHH and EROD induction values obtained by Sawyer and Safe[51] and Safe[52] are given in Table 5; TEF values derived from the relative activities are shown in Table 6. When ranking the PCB congeners in terms of AHH activity, it is possible to see the effects of small changes in the PCB structure on the activity. PCBs with less than three chlorine atoms (not included in Table 5)

Table 5 Summary of structure-function relationships for planar, mono-ortho and di-ortho substituted PCBs and 2,3,7,8-TCDD*

Congeners	*Cytochrome P-450 induction type*[a]	*EC_{50}*[b]		*Relative activity*[c]		*Receptor binding affinity*		*Toxicity*[e] *ED_{50}* ($\mu mol \cdot kg^{-1}$)
		AHH	*EROD*	*AHH*	*EROD*	*EC_{50}*[d]	*relative affinity*[b]	
Planar								
77	MC	$3.5*10^{-8}$	$8.9*10^{-8}$	$2.1*10^{-3}$	$2.0*10^{-3}$	$7.1*10^{-7}$	$1.4*10^{-2}$	
126	MC	$2.4*10^{-10}$	$2.5*10^{-10}$	$3.0*10^{-1}$	$7.2*10^{-1}$	$1.3*10^{-7}$	$7.8*10^{-2}$	0.95
169	MC	$6.0*10^{-8}$	$2.4*10^{-8}$	$1.2*10^{-3}$	$7.5*10^{-3}$			8.9
37	mixed							
81	mixed	$1.1*10^{-5}$		$6.5*10^{-6}$				
Mono-ortho								
105	mixed	$8.8*10^{-8}$	$1.2*10^{-7}$	$8.0*10^{-4}$	$1.5*10^{-3}$	$4.2*10^{-6}$	$2.3*10^{-3}$	1030
114	mixed	$9.7*10^{-7}$	$5.7*10^{-7}$	$7.4*10^{-5}$	$3.1*10^{-4}$	$4.1*10^{-6}$	$2.5*10^{-3}$	200
118	mixed	$1.2*10^{-5}$	$8.9*10^{-6}$	$6.0*10^{-6}$	$2.0*10^{-5}$	$9.1*10^{-6}$	$1.1*10^{-3}$	1550
123	mixed	$3.9*10^{-6}$	$1.1*10^{-6}$	$1.8*10^{-5}$	$1.6*10^{-4}$	$4.1*10^{-6}$	$2.5*10^{-3}$	2790
156	mixed	$2.1*10^{-6}$	$9.0*10^{-7}$	$3.4*10^{-5}$	$2.0*10^{-4}$	$7.1*10^{-6}$	$1.4*10^{-3}$	180
157	mixed	$7.1*10^{-7}$	$1.3*10^{-6}$	$1.0*10^{-4}$	$1.4*10^{-4}$	$4.7*10^{-6}$	$2.1*10^{-3}$	225
167	mixed	$1.3*10^{-5}$	$9.0*10^{-6}$	$5.5*10^{-6}$	$2.0*10^{-5}$	$1.6*10^{-5}$	$6.3*10^{-4}$	
189	mixed	$1.1*10^{-5}$	$7.8*10^{-6}$	$6.5*10^{-6}$	$2.3*10^{-5}$			
Di-ortho								
128	mixed							
138	mixed							
158	mixed							
166	mixed							
170	mixed							
137	PB							
153	PB		inactive			$7.9*10^{-5}$	$1.3*10^{-4}$	
168	mixed					$1.0*10^{-4}$	$1.0*10^{-4}$	
180	PB							
190	PB							
191	PB							
199	?							
205	PB							
TCDD	MC	$7.2*10^{-11}$	$1.8*10^{-10}$	1.0	1.0	$1.0*10^{-8}$	1.0	0.09

*Adapted from Parkinson and Safe[24] and Goldstein and Safe.[38]

[a]MC, 3-methylcholanthrene; PB, phenobarbital; ?, type of induction not known.

[b]*In vitro* EC_{50}: concentration (M) producing half-maximal induction of total activity.

[c]Relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin.

[d]EC_{50}: concentration (M) required to displace 50% of bound radio-labelled TCDD.

[e]*In vivo* ED_{50}, thymic atrophy, rat.

Table 6 Estimation of the TCDD equivalent concentration for the six most toxic PCB congeners in aquatic biota

Organism	*PCB No.:*	*77*	*105*	*118*	*126*	*156*	*169*
	Structure:	*33'44'*	*233'44'*	*23'44'5*	*33'44'5*	*233'44'5*	*33'44'55'*
Concentration in biota ($ng \cdot g^{-1}$)							
Dalls porpoise[a]		2.3	100	280	0.16	11	0.11
Bairds beaked whale[a]		1.3	4.9	47	0.37	23	0.24
Killer whale[a]		48	3000	11 000	3.70	1900	77
Herring gull eggs[b]				580		362	
Adouin's gull eggs[b]				1671		664	
Shags eggs[b]				1256		569	
Cod S. North Sea[c]				440			
Cod mid-North Sea[c]				110			
Cod N. North Sea[c]				54			
Mackerel oil[d]				143			
Cod liver oil[d]				456			
Eel[e]		0.1–3		14–290	0.01–0.2		9.10^{-5}–1.10^{-3}

	77		*105*		*118*		*126*		*156*		*169*	
	A[f]	*E*[f]	*A*	*E*	*A*	*E*	*A*	*E*	*A*	*E*	*A*	*E*
TCDD equivalent concentration ($pg \cdot g^{-1}$)												
Dalls porpoise	4.8	4.8	83	15	1.8	0.6	48	122	0.38	0.22	0.13	0.87
Bairds beaked whale	2.7	2.7	40	0.7	0.3	0.1	105	281	0.80	0.47	0.29	1.90
Killer whale	100	100	248	450	69	23	1110	2812	66	39	92	608
Herring gull eggs					3.6	1.2			13	7.4		
Adouin's gull eggs					10	3.5			23	14		
Shags eggs					7.9	2.6			20	12		
Cod S. North Sea					2.8	0.9						
Cod mid-North Sea					0.6	0.2						
Cod N. North Sea					0.3	0.1						
Mackerel oil					0.9	0.3						
Cod liver oil					2.9	1.0						
Eel	0.3–6	0.3–6			0.1–1.7	0.3–5.8	3.3–68	8–160			$<2.10^{-3}$	<0.015

[a]Tanabe *et al.*[25]; [b]Focardi *et al.*[53]; [c]De Boer.[54]; [d]Wells *et al.*[55]; [e]Dorgelo.[56]; [f]A = AHH, E = EROD.

are unable to fulfil the basic requirement for a toxic, planar molecule, viz. substitution at both para positions and at least two meta positions. Of the remaining eight PCBs with no ortho substitution only three appear to have AHH activity. These are PCB 77, PCB 126 and PCB 169. Bradlaw and Casterline[50] clearly demonstrated this by comparing the ED_{50} response for PCB 77 (10,250 pmol plate^{-1}) and the 3,5,3',5'-tetra-CB (PCB 80) which, with the loss of the ortho-meta vicinal hydrogens is inactive at 50,000 pmol plate^{-1}. In addition to data for these three PCBs, Safe[51] also reported AHH and EROD activity values for the eight mono-ortho substituted PCBs (cf. previous section). It is interesting to note that there is a factor of 10^5 difference in activity between the most active congener, PCB 126 and PCB 118. On the other hand there is a difference of a factor of approximately 10^{-5} in their relative concentrations in PCB industrial formulations and in the concentrations found in environmental samples.[14,54,57] The most toxic PCB 126, in turn, is some 50 times less toxic than TCDD, but again the relative concentrations found in the environment lead to a TCDD equivalent concentration of PCB 126 which is, in fact, greater than that of TCDD itself.[14,25,56,57]

The most widely measured PCB which can adopt an almost planar structure and is relatively abundant in most environmental compartments is the mono-ortho PCB 118. As an example, and in addition to the data published by Tanabe *et al.*[13] on the relative TEF values for sea mammals in the Pacific, the TEF values for PCB 118 and PCB 156 for sea bird eggs,[53] for PCB 118 in cod from the North Sea[54] and for eel[56] were calculated by us (cf. Table 6). A comparison with the TCDD equivalent concentrations in sea mammals[13] of the data for PCB 118 for both the Mediterranean sea birds and, especially, southern North Sea cod suggests that it is likely that the planar and mono-ortho substituted molecules are present at a detectable and, probably, toxicologically relevant level. Recently, this has been substantiated for the mono-ortho congener 105 as well.[26,58,59] Even the Certified Reference Material (CRM) cod liver oil[55] contains a significant TCDD equivalent concentration of PCB 118.

The implication from these data is that a wide variety of aquatic biota probably contain detectable quantities of the more toxic planar PCBs; if their AHH/EROD activities are additive, this can account for a significantly greater potential toxicity than contributed by either the PCDDs or PCDFs. It is only fair to add however that, recently, the additivity principle implicitly present in the TEF concept has been criticised on the basis of the dioxin-antagonistic activity of competitively binding less or non-effective congeners.[60]

2.4 Conclusion

PCBs—like chlorinated dioxins, dibenzofurans, azo(xy)benzenes and naphthalenes—are biologically active as a result of binding to a cytosolic receptor called the Ah receptor. The binding affinity appears to depend on structural properties of the congener, illustrating the stereospecificity of the receptor. The receptor is thought to regulate the synthesis of a variety of cytosolic enzymes. Toxicity presumably results from perturbations of the control of structural genes for a

number of proteins.[38] Therefore, structure-affinity relationships for the binding of PCBs to the Ah receptor can be used to predict the inducing activities and toxicity of individual PCB congeners. In addition, the known inducing activities and toxicity can be used in structure-activity relationships to further predict activities of untested congeners.

Cytochrome P-450 enzyme activity has been established now for several teleosts, and purified cytochrome P-450 isoenzymes from rainbow trout, cod, perch and plaice have been isolated.[81] These were used successfully in place of rat hepatoma cell cultures[24] to study the AHH/EROD-inducing activity in aquatic organisms. Such biochemical monitoring also needs to be developed to identify antibodies which may be used against the induction of cytochrome P-450 and employed in enzyme-linked immunosorbent assay (ELISA) techniques.[62]

3. DETERMINATION OF PLANAR CHLORINATED BIPHENYLS

Carrying the study of the toxic effects of individual planar PCBs from the laboratory—where relatively high concentrations are often used without the added complexity of the presence of other PCBs and/or halogenated organics—to the real environment has been hampered by the impossibility to completely separate and/or correctly identify and quantify these planar PCBs. As to the majority of the mono- and di-ortho substituted congeners, their concentrations are usually a few orders of magnitude higher than those of planar PCBs, which renders their determination less difficult. The limited availability of the pure planar congeners for calibration, and the problems connected with the analytical methodology have now largely been overcome and, while improvements are still needed, the basic techniques for the determination of the individual planar PCBs are available.

3.1 Sample size

Although the determination of planar PCBs follows the same general route as for the other, more abundant PCBs—viz. extraction, clean-up and group separation prior to the final determination by GC with either ECD or MS with multiple ion detection (MID)—the presence of these compounds in the environment at ultra-trace concentrations (1–10 $pg \cdot g^{-1}$) requires significant refining of the normal routine methods.

Kannan *et al.*[10,27] have determined the planar PCBs in several commercial Aroclor mixtures; they found 260–6100 $\mu g \cdot g^{-1}$ for PCB 77, 8.6–62 $\mu g \cdot g^{-1}$ for PCB 126 and 0.05–0.51 $\mu g \cdot g^{-1}$ for PCB 169. Using these data and the known total PCB concentrations of, for example, Aroclor 1254, it is possible to estimate the approximate range of the planar PCB levels in the various environmental compartments. Sea water in the open ocean may contain as little as 0.03–1.0 $ng \cdot l^{-1}$ of total PCB,[12] which would lead to as little as 0.001 $pg \cdot l^{-1}$ of the planar PCBs. This concentration is well below the detection limits (cf. below) and would require an initial sample size of 100,000 l in order to approach these limits.

Although techniques to obtain and process large samples volumes are available,[63] sample handling and solvent manipulation are certainly beyond the means of most, if not all laboratories.

Actually, in water a total PCB concentration of 5–50 $\mu g \cdot l^{-1}$ would be required in order to detect the planar congeners at a limit of detection (LOD) of approx. 0.05–0.5 $ng \cdot l^{-1}$ in a 1 l sample—the calculation being based on an absolute LOD of 1 pg, an injected volume of 1 μl and a final extract volume of 0.1–1 ml. This concentration of total PCBs would cause the water to be regarded as highly polluted.[64]

The size of a sediment sample required for analysis is, of course, also dependent on the degree of contamination. Relatively clean open sea sediments may have PCB concentrations in the range of 1–5 $ng \cdot g^{-1}$. At this background concentration of PCBs it may be necessary to take a sample size of up to 10 kg for extraction in order to meet the detection limits for the planar PCBs. However, there are numerous sites known to be contaminated with PCBs far in excess of these levels and, at e.g. 1000 $ng \cdot g^{-1}$ (which is a realistic level; cf. refs. 65 and 66) a sample size of 5–50 g would generally be sufficient to be able to detect traces of the planar PCBs.

Fish tissue from relatively uncontaminated waters, e.g., caplin, *Mallotus villosus*, from the northern North Sea/Icelandic waters, may contain as little as 10 $ng \cdot g^{-1}$ of total PCBs. The planar PCBs are unlikely to be detected at this low level of contamination, even with bulk samples of 100 g. However, species from moderately polluted waters where total PCBs in the tissue are present at levels of over 500 $ng \cdot g^{-1}$ will not present too many problems. In that case, it will be feasible to determine the planar congeners on samples of 10–50 g wet weight. The concentration of the PCB residues in biological tissue is positively correlated with the extractable lipid levels.[67] In can therefore be expected that species with a normally high lipid content, e.g., herring, *Clupea harengus*, and mackerel, *Scomber scombrus*, and most sea mammals will contain a relatively high body burden of the planar PCBs and can, therefore, be analysed relatively easily.

The range of the sample sizes required for the determination of the three planar congeners in the three main aquatic compartments are summarised in Table 7. From these data it is clear that the normal methods of sample preparation used with most PCB analyses[12] need to be improved significantly as regards their scale of operation.

A decrease in LOD can be obtained by the injection of larger volumes into the capillary GC column. Two developments are of interest in this case.[68] First, a retention gap can be installed at the inlet of the GC column, which permits the injection of volumes of up to 10, or even 100 μl. In the retention gap—a piece of uncoated, but deactivated capillary column—the sample is directly introduced (on column) or condensed (splitless) and it subsequently floods the non-coated area. When the solvent evaporates, the solutes migrate through the column and finally are cold-trapped on the analytical column. The length of the non-coated column required to encompass the flooded zone, which may not become a significant fraction of the total column length, restricts the total volume that can be applied.

A second possibility is to use a solvent purge technique, which preseparates

Table 7 Estimated sample sizes required to determine planar PCBs in environmental samples

Sample type	*Concentration* ($ng \cdot l^{-1}$ *or* $ng \cdot kg^{-1}$) *of*		*Minimum sample size required (LOD)*[a] *(l or kg)*	*Sample size for ideal detector response*[b] *(l or kg)*
	Total PCB	*Planar PCB*		
Clean open sea	10^{-1}	10^{-6}	10^{5}	5.10^{6}
"Harbour" water	10^{2}	10^{-3}	10^{2}	5.10^{3}
Sandy, open sea sediment	10^{3}–10^{4}	10^{-2}–10^{-1}	1–10	50–5.10^{2}
Estuarine sediment	10^{6}–10^{7}	10–10^{2}	10^{-2}–10^{-1}	5.10^{-1}–5
Low-lipid fish	10^{4}–10^{6}	10^{-1}–10	10^{-2}–1	5.10^{-1}–50
High-lipid fish/ (sea) mammal	10^{6}–5.10^{8}	10–5.10^{3}	2.10^{-4}–10^{-2}	10^{-2}–5.10^{-1}

[a]Assuming an absolute limit of detection of 1 pg and a final extract of 0.1 ml.
[b]Sample size required for a detector response in the middle of the linear range of the detector.

volatile material, including the solvent, from high-boiling solutes in the injector. To this end, a programmed temperature vaporiser (PTV) split inlet is required. The solvent evaporates together with low-boiling compounds during the initial split-open period, while the less volatile compounds remain cold-trapped in the injection liner. Next, the splitter is closed and the inlet is rapidly heated, allowing the remaining compounds to evaporate and enter the column. This technique can, of course, only be used when early eluting peaks are not of interest and requires a large difference in solvent and solute boiling points. Sample sizes of over 50 μl have been injected into a capillary GC column.[68] These techniques therefore allow an improvement of the LOD by a factor of between 10 and 50.

Finally, the LOD can be reduced substantially by using a micro reversed-phase LC column directly coupled to the capillary GC system through an interface consisting of an on-column injector and a retention gap. These systems have been used successfully for the enrichment of PCBs and pesticides from aqueous and sediment samples[69,70] at the ppt level, since they allow one to use injection volumes of up to 1 ml. Sample pretreatment may be much shorter when these systems are used, and the time of analysis can be reduced to the time of the GC run.[69]

In all the above cases, it is assumed that the LOD is determined by the inherent sensitivity of the analytical system, and not by the presence of interfering matrix components. Unfortunately, in actual practice, the opposite will often be the case.

3.2 Extraction and Clean-up of PCBs

The isolation of PCBs from an environmental sample and the removal of the bulk of co-extracted material can be achieved by a variety of well established and

published techniques; Erickson[12] gives a complete overview of the standard procedures for the determination of PCBs. The planar PCBs are extracted along with most other chlorinated aromatic trace contaminants. The physical treatment of the matrix at the extraction stage is important to ensure good solvent penetration into the whole sample. Liquid–liquid extraction is mostly used for the extraction of aqueous samples. In addition, preconcentration techniques have been developed to cope with the usually relatively low concentrations of PCBs in water. In particular, the trace enrichment by (pre)column liquid chromatography with on-line coupling to capillary gas chromatography is promising in this respect.[69] With sediment and biological tissue physical mixing, maceration and blending have been used. Muscular or sinuous tissue must be homogenised. Ultrasonic treatment and saponification with ethanolic potassium hydroxide have also been used to disrupt cellular or macromolecular material which may contain PCBs. The use of binary or tertiary solvent mixtures has been developed to aid the "wetting" of the whole sample and to aid the transfer of the PCBs into the less polar solvent. Most techniques require the removal of the more polar solvents, e.g., acetone or dichloromethane, prior to additional clean-up. An overview of some of the more established extraction techniques is given in Table 8.

Most analytical schemes developed for PCB analysis belong to one of two categories: those which are specifically designed for the determination of PCBs, and those which involve a multi-residue scheme to include the determination of trace contaminants other than PCBs.[12]

The specific PCB methods permit a more rigorous further treatment of the extract, because of the relative inertia of PCBs towards chemical agents. Saponification either before, during or after extraction is possible using ethanolic potassium hydroxide.[25,104,105] Saponification has been shown to be more effective than soxhlet extraction with non-polar solvents, in particular with lean tissue.[96] However, care should be taken to control the saponification conditions, particularly the temperature. Van der Valk and Dao[106] have reported the degradation of PCBs during alkaline hydrolysis and recommend that the reaction temperature should be kept below 70 °C. Saponification removes much of the biogenic co-extractable material and allows a greater concentration of the sample prior to liquid chromatographic treatment.

Concentrated sulphuric acid has also been used successfully to remove interfering co-extractants and organic macromolecules, viz. by oxidation, solvatisation and dehydration.[84,107,108] In most applications the extract is shaken with the concentrated acid and the degraded organic material dissolved in the acid. Lamparsky and Nestrick,[109] Smith *et al.*[110] and Tanabe *et al.*[25] have used a silica column impregnated with sulphuric acid (40%, w/w) which is considerably more efficient, and safer and easier to manipulate. These techniques have been used in the treatment of both sediment and biological tissue extracts, and serve to remove the bulk of the exogenous organic material.

Although saponification will remove most of the elementary sulphur and mercaptans, additional treatment may be necessary. Four main techniques have been published for the removal of sulphur present in sediment and sewage sludge extracts; these involve mercury,[88] sodium sulphite,[84] silver[111] or copper/copper

Table 8 Summary of the methods of extraction used for PCB-containing samples

Sample type	*Method*	*References*
Water	Liquid–liquid extraction, dichloromethane/hexane (15:85); study of extraction solvents	71,72
	Liquid–liquid extraction, followed by KOH saponification of papermill effluent	73
	Continuous liquid–liquid extraction	74
	Reduced emulsions combined with steam distillation, micro version	75
	XAD-2 column extraction	76
	Polyurethane foam	77
	XAD-2, XAD-4 (sea water, tap water)	78
	Tenax	79
	C-18 bonded-phase cartridges	80
Sewage sludge	Centrifuge, acetone/dichloromethane/hexane (2:15:83). Column elution, sludge mixed with anhydrous sodium sulphate eluted with acetone/hexane (20:80). Soxhlet, sludge and sodium sulphate extracted with dichloromethane/hexane (15:85)	81
	Centrifuge, acetone, centrifuge, sodium chloride (0.2 M) in phosphoric acid extraction acetone/hexane (1:3) extraction, hexane/diethylether/undecane (90:10:2) extraction. Sulphur removal. Potassium hydroxide treatment	84
Sediments, soils	Solvent extraction. Shake alternatively with acetone and hexane, triple extraction	82,83,84 85
	Sequential acetone and hexane	86
	Wetting dry sediment improves recovery from 30% to 85%	87
	Soxhlet acetone/hexane (10:90)	85
	Sonication. Hexane/dichloromethane (85:15) Nielson–Kruger steam distillation	89
Blood and serum	Rotating mixer, hexane	90
	Triple extraction, ethyl ether/hexane (50:50)	91,92,93
	Ethyl ether/hexane extraction and saponification with KOH in methanol	94
Fish tissue	Soxhlet extraction. Diethyl ether, diethyl ether/pentane (50:50)	95
	Comparison of different extraction methods	96
	Acetone (diethyl ether, hexane)	97
Shellfish	Petroleum ether, methanol, chloroform	98
	Comparison of column extraction methods	99
	Column chromatography: frozen tissue ground with sodium sulphate/sand	100,101
	Distillation: Nielsen–Kruger modified system	102,103
	Saponification; ethanolic KOH/hexane	104

wool.[110,112] Mercury does remove some interferences, but even repetitive treatment is insufficient for the total removal of sulphur.[113] Metallic silver has been shown to be successful when impregnated on silica columns.[111] Preliminary results of our group indicate, however, that drastic changes in the elution pattern of the chlorobiphenyls can occur in this case. Copper probably not only removes elemental sulphur but also mercaptans and disulphides. Although somewhat laborious, the sodium sulphite treatment is now generally preferred in sulphur-rich sediment analysis.

When other chlorinated organics, such as, e.g., chlorinated pesticides, have to be determined as well, less destructive methods have to be used. Non-destructive techniques have centred around gel permeation chromatography (GPC),[114] to remove lipid material, and adsorption chromatography using magnesium silicate (Florisil), alumina and/or silica, and combinations of the latter two.[115] Reviews[12,116] of these techniques primarily cover the work-up of modest amounts of tissue (< 20 g) or sediment (< 50 g) extract. In most applications the lipid co-extractant loading of the column will be the limiting factor and some modification may be required to cope with the increase in sample size necessary for planar PCB determination. In most cases GPC is limited to 1–2 g of lipid, and the conventional adsorption chromatography columns do not operate efficiently above 200 mg of lipid. Increasing the dimensions of these techniques will result in a large throughput of solvents and adsorbents, introducing high costs and higher risks of contamination.

Instead of trying to adapt the organochlorine/pesticide "master analytical schemes" and/or the dimensions of commonly used chromatographic clean-up columns, it may be more cost-effective to develop methods specifically aimed at the separation of planar PCBs from other congeners, based on their structural properties, as will be discussed in the following section.

3.3 Sample Pretreatment for and Separation of Planar PCBs

The separation of planar PCBs, and also PCDDs and PCDFs and their methoxy derivatives[117] from non-planar PCBs has been achieved primarily by the use of various crystalline forms of active carbon as column material in column liquid (adsorption) chromatography. The main exception was an early attempt to separate PCB 77 and PCB 169 in Aroclor mixtures on Florisil.[118] However, activated carbon has a number of specific advantages, viz. a greater selectivity for planar molecules (due to the graphitic structure) and a more reproducible behaviour, which is determined by the activity of the adsorbent; besides, it is less prone to contamination by sample constituents since it combines a high capacity with a low sorption for endogenous co-extracted material.

Initial experiments with carbon were aimed not so much at the isolation of planar PCBs from non-planar ones, but rather at the separation of organochlorines from PCBs or the separation of PCDDs and PCDFs from PCBs and other major apolar organochlorines in environmental samples (cf. ref. 12 and references therein). Teichmann *et al.*[119] used a slurry-packed 90 × 6 mm ID column using 50–200 mesh charcoal after an initial separation over alumina. Soil extracts

were fortified with PCBs and chlorinated pesticides at a level of 1–3 $\mu g \cdot kg^{-1}$ (pesticides) or 70 $\mu g \cdot kg^{-1}$ (PCBs). The pesticides were eluted with acetone:diethylether (1:3, v/v); the PCBs were eluted with a subsequent portion of benzene. Recoveries were between 80 and 100%, which is quite acceptable.

Chau and Babjak[85] used carbon foam to separate PCBs from chlorinated pesticides in lake sediment extracts. The columns were prepared from chopped polyurethane foam coated with a carbon (Norti C-170) slurry in chloroform. The technique used cyclohexane to remove most of the chlorinated pesticides and, next, benzene to elute the PCBs, p,p'-DDE and α-HCH. Since there was no further separation of PCBs—although the potential to do so was inherent in the method—the technique as such offered little advantage over conventional adsorption chromatography using alumina or silica. The main disadvantage of the foam support was the inherent loss of the foam structure with the use of the more polar and chlorinated solvents.

However, already in 1974, Jensen and Sundström[120] used a 200 × 15 mm ID activated charcoal column (Darco G-60) to separate four groups of PCBs in Clophen technical mixtures, viz. (1) tetra-ortho chlorines, and (2) tri-ortho chlorines, which were both eluted with tetrahydrofuran, and (3) mono-or di-ortho chlorines and (4) non-ortho chlorines, both of which were eluted with benzene.

Huckins *et al.*[121] used activated charcoal dispersed on polyurethane foam to determine the four planar PCBs in Aroclors. The columns (100 × 10 mm ID) contained AMOCO PX-21 dispersed on the foam, 15 wt.%. Stalling *et al.*[114] used the same type of activated charcoal and column size with a loading of 1.75 g of PX-21 to elute the PCBs into five fractions, viz. tetra-, tri, di-, mono- and non-ortho PCBs, using a step gradient of toluene-cyclohexane (2:98 to 100:0).

Since polyurethane foam is not fully reliable because it breaks down in polar solvents, glass fibres were employed as an alternative support.[110] A series of columns were used in a semi-automatic configuration to extract the PCBs from fish tissue—previously dried over sodium sulphate—with dichloromethane:benzene (1:1). The extract is passed over potassium silicate, silica, caesium silicate, silica and, finally, activated charcoal (Amoco PX-21) deposited on glass fibres. The planar molecules are retained on the carbon column and later recovered by backflushing with toluene. A similar system using carbon fibres has been used fully automatedly under microprocessor control.[112]

Shaw and Connell[123] used a 20/80 charcoal (5–20 μm)/Celite packing in a 30 cm high-performance liquid chromatographic (HPLC) column to obtain adsorption characteristics of PCB congeners onto a planar surface. They found a moderate correlation between the HPLC elution time on charcoal and the steric properties of the analytes.

Recent improvements in carbon chromatography have led to greater resolution between classes of planar compounds, such as PCDDs, PCDFs and planar PCBs, at the ultra-trace level. Tanabe *et al.*[25] have reverted to a more simple approach of saponifying the tissue with ethanolic potassium hydroxide at the extraction stage, then applying the partitioned extract to a 35 × 5 mm ID activated carbon column. The sample is applied to the column in hexane and the bulk of the organochlorine

residues and non-planar compounds are eluted with dichloromethane:hexane (1:5). Benzene:ethylacetate 1:1 is used to elute the planar PCBs and, finally, toluene is used to elute the PCDDs and PCDFs. The recovery of PCBs 77, 126 and 169 was over 90% at a spiking level of about $10\,ng \cdot g^{-1}$, and over 60% at a spiking concentration of $0.1\,ng \cdot g^{-1}$, which is the planar PCB level in blubber.

Huckins *et al.*[57] have extended the activated carbon-glass fibre method[110] to specifically separate planar PCBs from the sample matrix and the PCDDs and PCDFs. After the sample is cleaned up on sulphuric acid:silica and silica, the extract is applied to the activated carbon dispersed with glass fibres in a glass column. The tetra-ortho chlorine congeners are eluted with dichloromethane:cyclohexane 1:1, the tri-ortho PCBs with dichloromethane, and the remaining non-planar PCBs with 20 ml of benzene:methanol:dichloromethane (1:2:7). Finally, the planar PCBs are removed from the column by reverse flow elution with toluene.

The methods described above exhibit acceptable recoveries and relatively large sample capacities. However, they are laborious and require much solvent (for example, the elution scheme used by Tanabe *et al.*[25] requires solvent amounts of more than 1200 ml for one sample) and expensive equipment (e.g., multicolumn switching valves, backflushing apparatus, columns). Miniaturisation of the activated carbon clean-up has been reported recently.[124] Disposable minicolumns containing only 1 mg of activated carbon mixed with 20 mg of silica were successfully used for the isolation of PCDDs and PCDFs in a multi-stage clean-up procedure. Although the performance of this system was not evaluated for planar PCBs, it may well be successful for these congeners.

Creaser and Al-Haddad[125] have reported the use of porous graphite carbon (PGC) in a HPLC system. The PGC has a mean particle size of $7\,\mu m$, a surface area of $150\,m^2 \cdot g^{-1}$ and a mean pore volume of $2.0\,cm^3 \cdot g^{-1}$. It is currently the only graphitic material available which has the ability to withstand the back-pressure generated by HPLC and was specifically developed by Knox *et al.*[126] A 50×4.7 mm ID column using n-hexane as eluent can elute the organochlorine pesticides and the ortho-substituted PCBs in an initial 10 ml of hexane. The non-ortho PCBs are then eluted with a further 90 ml of hexane. The PCDDs and PCDFs are removed by reversing the flow and back-eluting with a further 200 ml. At a flow rate of $5\,ml \cdot min^{-1}$, each analysis takes some 60 min. This time may be reduced by back-flushing with toluene. However, the advantage of the single-solvent system is that the column performance remains constant over a longer period of time. Besides, n-hexane is less expensive at the level of purity required and the use of toxic solvents such as benzene is obviated.[25,110] The recoveries of the PCDDs and the PCDFs are 53–85%, but this was from fortified soil extracts and may not reflect the true efficiency of the system. The main disadvantage of PGC seems to be its relatively low capacity,[125] which necessitates prior removal of the bulk of co-extractants, for instance by adsorption chromatography.

Summarising the above, we can state that activated carbon and other types of graphitic carbon have been shown to possess a specific selectivity towards planar molecules, which can be used for the separation of planar chlorinated aromatics from non-planar congeners and related products. An example of a typical elution pattern is given in Figure 2. The selectivity is governed by (1) the interaction

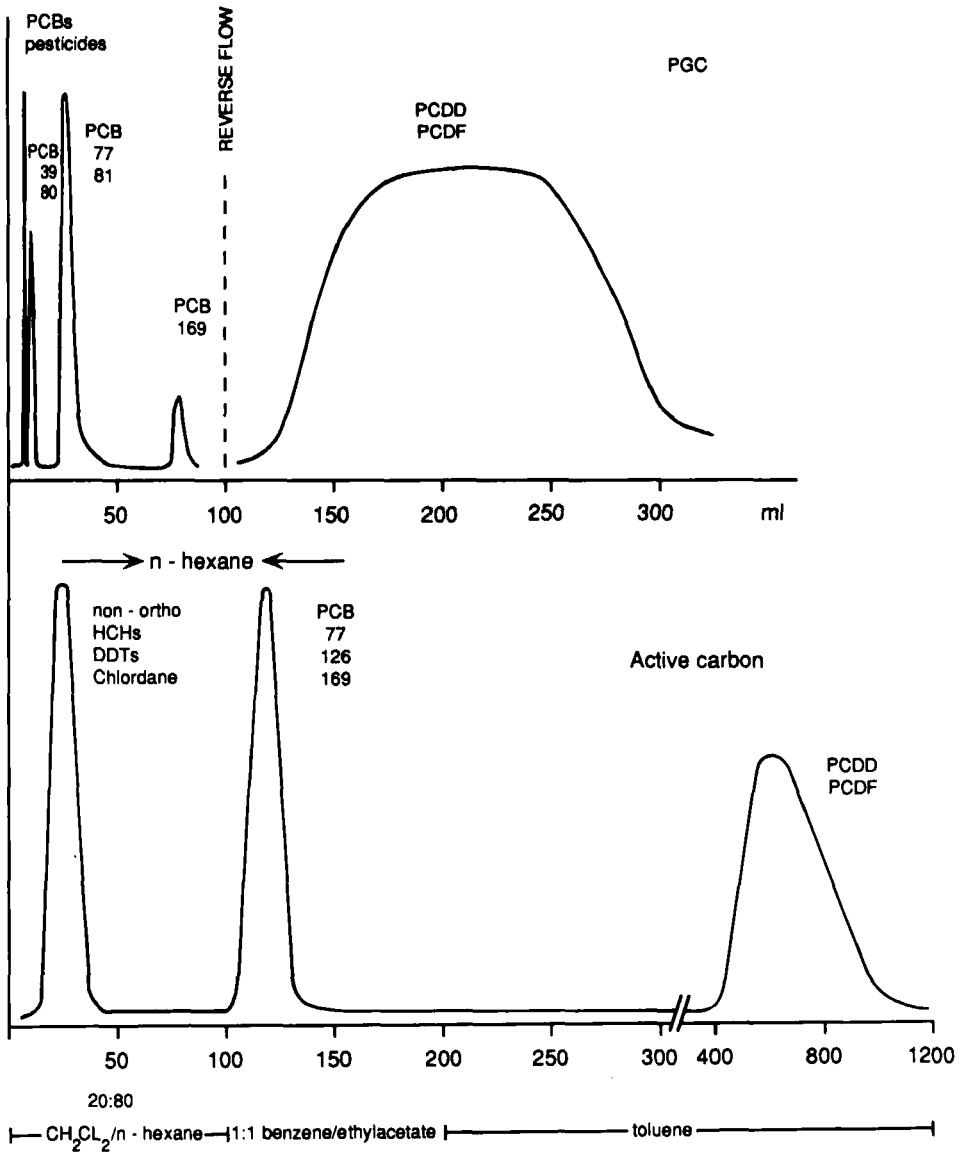

Figure 2 Carbon chromatographic separation of non-ortho PCBs and pesticides from planar PCBs, PCDDs and PCDFs. Adapted from Creaser and Al-Hadded[125] and Tanabe *et al.*[135] PGC: 50 × 4.7 mm I.D. HPLC column packed with porous graphitic carbon; eluent; n-hexane, 5 ml/min, back-flush from 100–300 ml; detection: 0–100 ml, UV 254 nm; 100–300 ml, GC-ECD. Active carbon: 35 × 5 mm I.D. adsorption chromatographic column filled with active carbon (charcoal); eluents: see ordinate, flow ca. 3 ml/min; detection: GC-ECD. Abbreviations: HCHs: hexachlorocyclohexanes, DDTs: dichlorodiphenyltrichloroethanes and metabolites.

between molecules which have a planar configuration (e.g., PCDFs, PCDDs, polychlorinated biphenylenes) or can easily adopt a planar configuration (such as the planar PCBs), and the (planar) graphitic surface,[125,126] and (2) the number of electronegative substituents on the biphenyl skeleton.[114,125] These specific properties of carbon thus enable the separation of planar PCBs, not only from non-planar PCBs, but also from PCDDs and PCDFs.

The most recent development to be mentioned is the use of a 2-(1-pyrenyl)ethyldimethylsilyated silica column for the successful HPLC isolation of both planar and mono-ortho congeners from technical PCB mixtures.[206]

3.3 Identification and Quantitation of Planar PCBs

The arrival of high-resolution capillary GC provided the possibility to identify and quantify individual PCB congeners. As the identification of individual congeners became more and more urgent in the past decade, the analytical methodology for PCBs gradually shifted from the use of packed column to capillary column chromatography. As a result, in several European countries monitoring of selected congeners took the place of monitoring "total PCB" concentrations in environmental programmes.[55,127] In West-Germany and in the Netherlands the determination of individual congeners is statutorily regulated for control of waste mineral oils.[128,129] A set of six or seven congeners is habitually used for monitoring, their selection being based on abundance, resolution, response and availability as a standard, with resolution probably being the weak spot, even in HRGC analysis (see also below).

In the initial sets of selected congeners no planar congeners, and only one of the mono-ortho congeners, viz. PCB 118, were present. Recently, the above selections have been criticised with regard to the resolution of some of the selected congeners[130] and their purity,[131] and because toxicity has not been included as a criterion. Moreover, their representativity for a diverging variety of sample types and commercial PCB mixtures (cf. Figure 3) is highly questionable.[132] As a result, new selections, in which resolution[130] and toxicity[4,133] have been among the major criteria, have now been proposed by several authors. As for the resolution criterion, its mere dependence on the use of one particular stationary phase renders it unsuitable for other stationary phases. This problem can only be resolved by standardisation of the stationary phase, which is highly unlikely for commercial reasons. Therefore, the extensive study carried out by Mullin *et al.*[3] for a SE-54 column need to be repeated for other commonly used commercially available capillary columns. For the rest, the selections proposed by Jones[4] and McFarland and Clarke,[133] which both cover a sufficiently wide range of chlorination, will suffer from the costly requirement to analyse 32–36 individual PCB congeners, among which also several poorly resolved congeners are present.

The identification of individual PCB congeners has to be done by firstly comparing their relative retention times in the chromatogram of the sample extract and that of the (individual) standard (mixture). Therefore, each congener should appear as a single peak. Subsequently, quantitation is carried out by comparing the peak heights or areas in both sample and standard solution. The use of

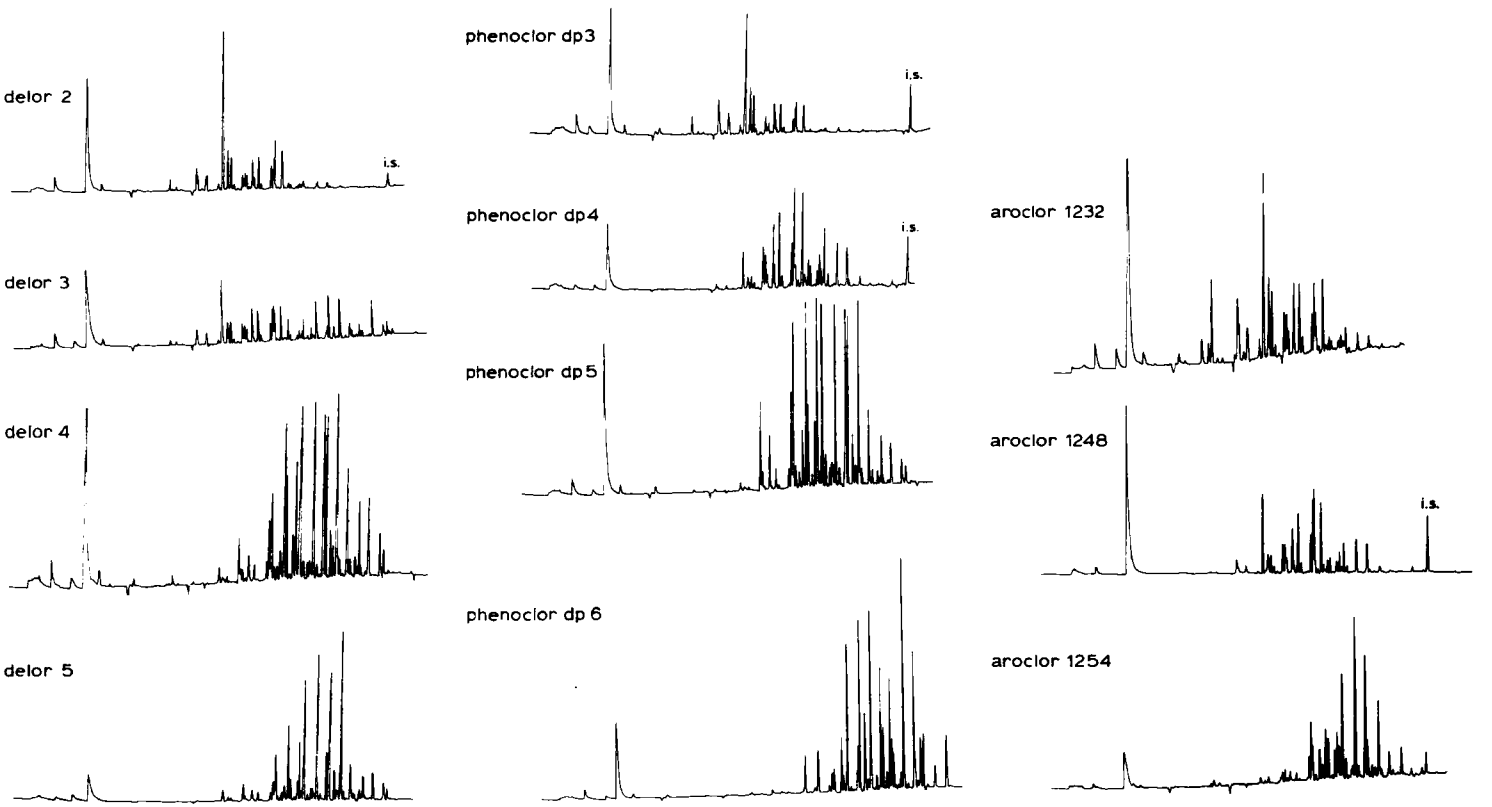

Figure 3 HRGC-ECD chromatograms of three Delor PCB mixtures, manufactured in Czechoslovakia, four Phenoclor PCB mixtures manufactured in France and three Aroclor PCB mixtures manufactured in the U.S.A. Note the similarities (compare, e.g., Delor 2—Phenoclor DP3—Aroclor 1232) and—more importantly—the differences (e.g., Delor 3, or Delor 5—Phenoclor DP5) between the PCB patterns of these commercial PCB mixtures which have been applied in practice and which therefore can be found in non-degraded or metabolised form in environmental matrices.

internal standards has been reported to be essential in this type of PCB congener analysis.[55].

Mullin *et al.*,[3] after having synthesised all individual compounds, have succeeded in separating 187 of the 209 PCB congeners on a 50 m SE-54 coated capillary column, using an ECD. Only eleven pairs of compounds, viz. 94/61, 70/76, 95/80, 60/56, 145/81, 144/135, 140/139, 133/122, 163/160, 202/171 and 203/196, exhibited identical retention times. In actual practice of individual congener analysis, however, resolution is usually somewhat less, causing overlapping peaks, even in the case of some of the above mentioned selected congeners. An extensive discussion of these problems has been given by Duinker *et al.*[130,134] Although the application of GC-MS can assist in identifying the presence of coeluting congeners with different chlorine numbers, it cannot do so in the case of congeners having the same number of chlorines. Moreover, in the case of the coelution of congeners with relatively low concentrations—as will very often be the case with, e.g., the planar PCBs—GC-MS is much less efficient.[130]

Two methods have been proposed to overcome the problems of resolution. First, a preseparation can be achieved according to the methods described in the preceding section. Once the planar congeners have been separated from the non-planar ones, their identification and subsequent quantitation by GC-ECD (or GC-MS) will not suffer from interference any more, and the only remaining problem may be the limit of detection. For example, Tanabe *et al.*[135] analysed the planar PCB fraction by GC-ECD and GS-MS following separation by carbon column chromatography. The three most toxic congeners, viz. the PCBs 77, 126 and 169, were determined at parts per trillion levels in biological samples and confirmed by MS monitoring of the m/z ratios 290:292 for PCB 77, 324:326 for PCB 126 and 358:360 for CB 169 (see Figure 4).

Alternatively, one can use two capillary GC columns with different polarity for the analysis of the PCB-containing extracts, either by simultaneous[55,129] or by multidimensional GC-ECD.[130] Of the latter two, only the multidimensional GC (MDGC) approach has been used for the separation of the planar congeners.[134,136] Since the latter are normally present as trace components in the sample extract, an almost complete resolution is essential to determine them. In the MDGC system, the eluate of the first column is sent to the ECD, where it appears interrupted during several intervals. During those intervals, the eluate is allowed to pass through a second column of different polarity and subsequently to a second ECD, which reflects the separation of the cuts as achieved by the combination of the two columns. A satisfactory separation was obtained for the planar PCBs using a SE-54 and a OV-210 capillary column in a MDGC system,[134] which allows the identification of a planar congener even when its relative contribution to the non-separated peak from the first column is as low as 0.01%. The use of a liquid crystal capillary column[137] as the second column in a MDGC system has also been shown to be successful in separating planar congeners from non-planar ones.[136] The enhanced separation power of liquid crystalline phases for rigid molecules is based upon molecular geometry;[137] it may be a promising improvement for the determination of planar PCBs and their mono-ortho congeners. Although heart cutting and further separation on a second

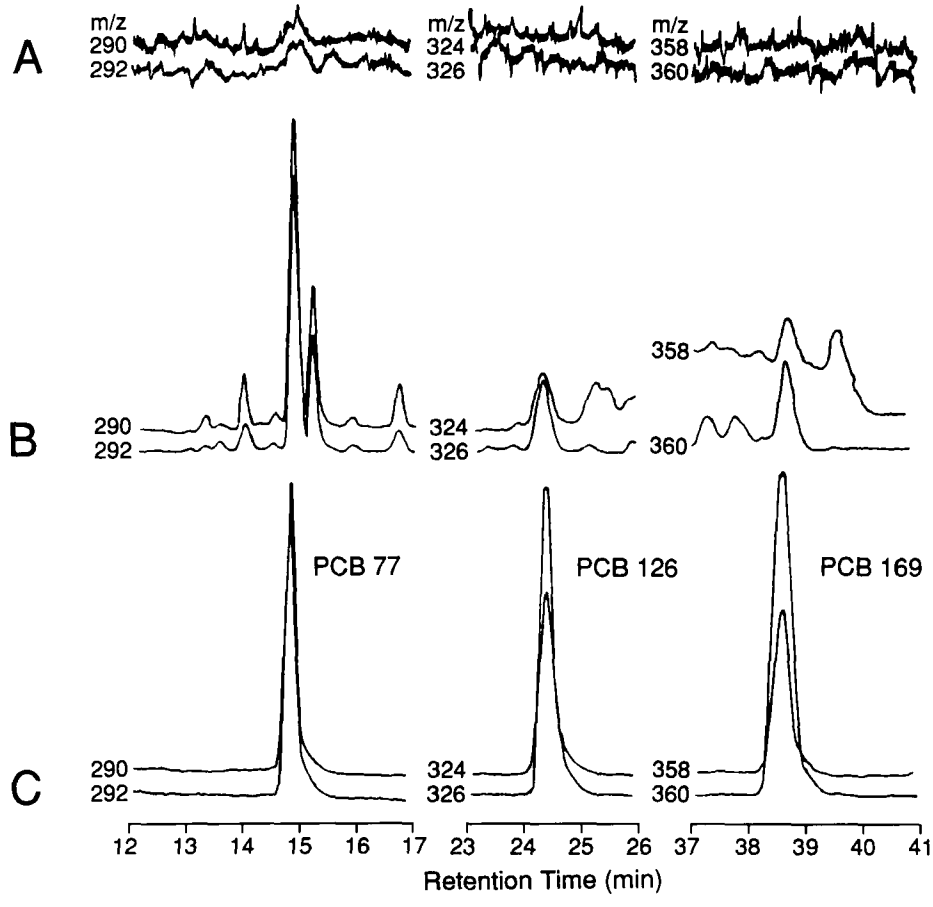

Figure 4 Mass fragmentographic analysis of planar PCBs in solution of porpoise blubber extract (B), and standard solution (C); (A) blank solution. Fragmentogram C was obtained by an injection of 5 μl of a standard mixture containing 230 pg PCB 77, 230 pg PCB 126 and 430 pg PCB 169. 10 μl of a concentrated hexane extract of blubber of finless porpoise were injected to obtain B. The blank was fragmentogrammed at 100-fold higher sensitivity. Adapted from Tanabe *et al.*[135]

column is ideal for identifying purposes, difficulties remain for quantitation as the internal standard(s) generally are not transferred to the second column, or require a second heart cut. Therefore, the standard addition technique has to be applied in MDGC. A major drawback of the MDGC technique is its time-consuming nature, which, in combination with standard addition, will probably limit its use to non-routine analytical programs.

3.4 Prediction of GC Retention Data

The non-availability of all individual congeners even in the recent past, and the high cost of obtaining all congeners as a standard today, is one of the main causes

of the lack of data on planar and mono- and di-ortho substituted congeners in environmental samples. One possibility to overcome this problem is the prediction of retention characteristics of individual PCBs from molecular-structure-based descriptors. Chromatographic retention has been predicted from such descriptors for various classes of compounds (see, e.g., refs. 138–140). Robbat *et al.*[141] have shown that two different models, one based on molecular connectivities[139] and the other on chlorine-position descriptors, can be used for the precise prediction of relative retention times or retention indices of all individual PCB congeners on various capillary columns, using between 35 and 40 standards. These predictive models can possibly be used for retrospective analysis of previously reported chromatograms. They may also be used for better identification of PCBs in current analytical programs. Finally, they can help in the selection of the optimum stationary phase when a particular set of congeners has to be fully resolved for proper identification and quantitation.

3.5 Conclusion

Only a few methods have been presented in the literature for the determination of planar PCBs in environmental samples. They are all rather laborious and time-consuming, whether in the isolation of the planar congeners from non-planar PCBs, PCDDs, PCDFs, etc. by carbon chromatography, or in the analysis of the fraction containing all PCBs by means of MDGC. None of the methods has been tested extensively with regard to accuracy. For several of the mono- and di-ortho analogues, viz. PCBs 105, 114 and 189, single-column HRGC can be sufficient for identification and quantitation in a fraction containing all PCBs. Others, however, e.g., PCBs 118, 123, 138, 156 and 157, have closely coeluting congeners,[134] and therefore require MDGC or GC-MS, or a preliminary group separation. For the planar congeners, the usually very low concentrations found in environmental samples pose an additional problem, for which trace enrichment and the processing of large-size samples need to be further developed. LC-GC systems offer interesting possibilities here, with regard to trace enrichment as well as analysis time. The latter compare favourably with MDGC, since the total analysis time in LC-GC is equal to the period of GC analysis. This is so because the trace enrichment is carried out during the GC run time of the previous sample.[70] Until now, negative chemical ionisation-MS has only been used in very few instances for the analysis of PCBs (see, e.g., refs. 142, 143); in the longer run, its more frequent use may well lead to improved limits of detection. Advanced MS systems (e.g., MS-MS) may also lead to improvements in the future, although their routine application cannot be expected in the next few years, due to the high costs involved.

As the need for accurate methods for the determination of planar and mono- and di-ortho PCBs is likely to increase because of the toxic potency of these congeners, intercalibration programs for these PCBs should be undertaken as soon as possible. Besides, the availability of the pertinent standards should be improved, and reference materials with certified levels of planar PCBs should be made available.

4. SOURCES AND OCCURRENCE OF PLANAR, MONO-ORTHO AND DI-ORTHO CHLORINATED BIPHENYLS

Polychlorinated biphenyls in the environment almost without exception originate from the use of technical mixtures of PCBs or commercial formulations containing such mixtures.[2] The composition of technical PCB mixtures has been studied extensively.[1,5-7,9,10,21,27,120,134,144-150] It is surprising that in the quantitative analyses of the same technical mixture the reported weight percentages of even major congeners nevertheless sometimes significantly differ from one paper to another. For example, for the composition of Aroclor 1260, Duinker *et al.*[134] and Safe *et al.*[21] reported 1.7 and 0.5 wt.%, respectively, for PCB 118, 9.6 and 11.4 wt.% for PCB 153 and 1.3 and 0.4 wt.% for PCB 156. This certainly is not only due to batch-to-batch differences, but also reflects the difficulties with resolution and identification. In Table 2 the composition of several technical PCB mixtures was given for congeners present for over about 2%. A complete literature search reveals that, except for PCB 166, all planar congeners and the mono- and di-ortho substituted congeners with mixed-type induction (cf. Table 4) have been reported to be present in at least one technical mixture.[4] Some notable differences also exist between the reported values for the planar congeners. Relevant results are given in Table 9. For the three planar congeners concentrations of less than one to several thousands of $\mu g \cdot g^{-1}$ are reported in PCB mixtures (cf. Table 9 and preceding section). It is therefore apparent that the planar congeners occur as only minor constituents in commercial mixtures.

In the natural environment, highly similar congener patterns are usually found within each compartment.[151] Between the compartments, however, the patterns often differ significantly, depending on the physico-chemical properties of the individual PCBs, the biokinetic routes (uptake, elimination and metabolism) and the degradation and decomposition kinetics.[151,152] Two major structural factors determine the environmental fate of PCBs, viz. the degree of chlorination and the position of the chlorine substituents. These factors, together with species characteristics—in particular the differences in basal levels of certain isozymes of cytochrome P-450 from species to species—also govern the biotransformation of PCBs.[153]

The lower chlorinated congeners are relatively abundant in the dissolved aqueous phase, whereas the higher chlorinated ones abound absorbed to particulate matter and sediments. Major components of technical PCB mixtures—e.g., PCBs 28, 110, 118, 138, 153 and 180—will almost always be found as major constituents of the total PCB content in abiotic samples. In biota, however, due to metabolic breakdown, significantly different—but specific—congener patterns can be observed for each species.[4] Planar PCBs are highly accumulative in lower organisms.[154] As to the selective accumulation of the planar PCBs and the mono-ortho congeners in higher animals contradictory results have been reported. Norstrom *et al.*[58] found similar ratios of PCBs 126/153 and 105/153 in Canadian birds and mammals compared to those in Aroclor mixtures. In Finnish wildlife, however, Paasivirta *et al.*[155] observed significantly higher enrichment rates from fish to seals and birds for PCBs 77, 126 and 169 compared to the average rate for

Table 9 Presence of planar and mono-ortho substituted PCB congeners in several commercial mixtures ($\mu g \cdot g^{-1}$)[a]

Congener	*Aroclor*			*Clophen*				*Kanechlor*			
	1242	*1254*	*1260*	*A30*	*A40*	*A50*	*A60*	*300*	*400*	*500*	*600*
Planar[b]											
77 D	5000	<100	<100	3900	6600	—	—	nd	nd	nd	nd
K	5260	645	270	nd	nd	nd	nd	4290	8040	1690	969
81 D	<100	<100	<100	1500	—	—	—	nd	nd	nd	nd
126 D	<100	<100	<100	—	—	800	4600	nd	nd	nd	nd
K	19	40	5	nd	nd	nd	nd	18	68	28	5
169 D	<100	800	500	—	—	1000	200	nd	nd	nd	nd
K	<0.08	0.6	<0.08	nd	nd	nd	nd	—	0.4	0.6	—
Mono-ortho[c]											
105	3300	2.10^4	800	4900	1.10^4	2.104	1200	5500	2.10^4	2.10^4	4500
114	—	—	—	600	1300	—	—	nd	nd	nd	nd
118	2.10^4	8.10^4	1.10^4	4200	2.10^4	1.10^5	2.10^4	7700	3.10^4	6.10^4	7900
123	—	9300	—	5500	5600	9300	—	nd	nd	nd	nd
156	1300	2.10^4	1.10^4	600	2300	1.10^4	1.10^4	640	1200	9900	5000
157	—	200	700	—	—	310	240	nd	nd	nd	nd
167	—	500	1500	—	—	500	4500	nd	nd	nd	nd
189	—	—	3500	—	—	—	4000	nd	nd	nd	nd

[a]For PCBs 77, 126 and 169 the values reported by Duinker *et al.*[134] (D) and by Kannan *et al.*[10] (K) are given (cf. text). The remaining data were taken from these references and from Kannan *et al.*[27] Figures over 10000 have been rounded off; for di-ortho congeners: see Table 2.

[b]—, below detection limit of 100 (Clophens; Duinker *et al.*),[134] 0.08 $\mu g \cdot g^{-1}$ (Kanechlors; Kannan *et al.*),[10] or as indicated by the authors (Aroclors; both references); nd, not determined.

[c]—, below detection limit of 100 $\mu g \cdot g^{-1}$.

Table 10 Occurrence of planar, mono-ortho and di-ortho PCBs in the environment[a]

PCB no.	*Presence in*					
	water	*sedi-ments*	*aquatic ecos.*	*terrestr. ecos.*	*human*	
					fat	*milk*
37	−	+	+	−	−	−
77	−	−	+	−	+	−
81	−	−	+	−	−	−
105	−	+	+/++	−	+	++
114	−	−	+	−	−	−
118	+	++	++	++	++	++
123	−	−	−	−	−	−
126	−	−	+	−	−	−
128	+	+	+	−	+	+
137	−	+	+	−	−	+
138	++	++	++	++	++	++
153	+	++	++	++	++	++
156	++	+	+	−	+	++
157	−	+	+	−	−	−
158	++	+	+	−	−	+
159	−	−	+	−	+	−
166	−	−	−	−	−	−
167	−	+	+	++	+	−
168	−	−	−	−	−	−
169	−	−	+	−	−	−
170	−	+	++	−	++	++
180	−	++	++	+	++	++
189	−	+	+	−	+	+
190	−	−	−	−	−	−
191	−	+	+	−	−	+
194	−	+	+	−	−	+
205	−	+	+	−	−	+

[a] −, not identified or not reported; +, positively identified; ++, component reported to be present in samples in excess of 2% of total PCB content.

all PCBs. More data are necessary to elucidate this controversy, since its importance for the understanding of the ecotoxicological meaning of the biochemically active PCBs is obvious. It would also be interesting to obtain similar data for other mono- and for di-ortho substituted congeners. Despite these contradictory findings, the ecotoxicological threat of the planar and the mono- and di-ortho congeners seems to be commonly accepted.

In the following sections an overview will be presented of the levels of planar and mono- and di-ortho congeners in various environmental compartments and in humans. Only reports using capillary GC-ECD or GC-MS have been considered. Table 10 presents a summary of the presence of planar and mono- and di-ortho congeners in environmental compartments and humans.

4.1 Water

Very few reports exist on the determination of individual congeners in natural waters and oceans, and none report on the less abundant planar PCBs. In general, very low concentrations of total PCBs are reported.[12] Due to the hydrophobic character of PCBs only trace levels will be found in the dissolved phase. The PCBs tend to adhere to particulate matter and most of this bound PCB will deposit in sediments or bioconcentrate through one of the aquatic food webs.

The overall PCB congener pattern in solution tends to deviate significantly from the patterns found in most commercial mixtures as well as in sediments or biota, presumably due to the differences in solubility between particular congeners.[156] As a consequence, the PCB pattern in water exhibits a shift towards the lower chlorinated congeners.[157,158] The following mono- and di-ortho PCBs have been reported in marine and freshwater systems: 118, 128, 138, 153, 156 and 170. In marine waters the individual concentrations of the mono- and di-ortho substituted congeners range between 0.01 and $0.1\,ng \cdot l^{-1}$.[158] In rivers the individual concentrations of the mono- and di-ortho congeners are between 0.1 and $50\,ng \cdot l^{-1}$.[145,157,159] In one sample, for the planar PCB 77 a concentration of $28\,ng \cdot l^{-1}$ was reported,[145] which is a finding of particular interest.

4.2 Sediments and Particulate Matter

In sediments, a number of di-, tri- and tetrachlorobiphenyls is found in relatively high concentrations, compared to biotic samples or commercial mixtures, where higher chlorinated congeners generally prevail. In North Sea sediments, Boon *et al.*[160] found a shift in PCB pattern towards higher chlorinated congeners when going from the open sea to the Dutch coast. The sediment samples taken in the vicinity of the coast not only contained 10-fold higher concentrations, but also showed tetra- to heptachlorobiphenyls predominating, whereas in offshore sediments di- to pentachlorobiphenyls prevailed. This may be due to two causes: (1) open sea sediments reflect the atmospheric deposition of PCBs, where more volatile congeners prevail, and (2) coastal sediments possibly reflect local, recent discharges, where selective uptake of higher chlorinated congeners by biota has not yet taken place. The mono- and di-ortho congeners in coastal and estuarine sediments around the Netherlands included the PCBs 105, 118, 128, 138, 153, 156, 157, 167, and 170, with typical concentrations of between 0.05 and $5\,ng \cdot g^{-1}$ (dry weight).[8,160,161] Most of these compounds were also found in invertebrates from the area. Planar PCBs were not determined or were below the detection limits. In polluted sediments and sludges the concentrations of total PCBs can be as high as $1000\,\mu g \cdot g^{-1}$ (dry weight),[65,162] with individual congener concentrations of between $0.1–50\,\mu g \cdot g^{-1}$.

4.3 Biota

In 1980, Zell and Ballschmiter[163] presented a list of the major PCBs occurring in environmental biotic samples. On the basis of their data the authors proposed that

"recalcitrant" PCBs tend to have a 4,4'- or a 3,4',5-substitution pattern. These PCBs are metabolised to a considerably lesser extent by the lower members of the aquatic community and are biomagnified as they pass to cetaceans, pinnipeds and fish-eating birds through the food chain. Such congeners will therefore be found in relatively high concentrations in species at the end of food chains compared to the technical formulations. On the other hand, fish feeding on phytoplankton will show only a small deviation from the patterns of the technical mixtures, reflecting minimal environmental degradation.

Such biotransformation has been studied in aquatic systems to determine the PCB patterns in aquatic organisms and the biological half-lives of individual congeners. For example, Boon[164] has shown that the uptake of a mixture of PCBs by sole, *Solea solea*, is proportional to the relative concentrations of the congeners in the mixture. The elimination, however, depends on the molecular structure of each congener. In general, lower chlorinated PCBs are more easily (bio)degradable. Furthermore, PCBs containing two vicinal unsubstituted positions in the biphenyl skeleton are more readily degraded than their corresponding isomers.[33,165]

Recently, the occurrence of PCB congeners in biota has been received by Jones[4] and McFarland and Clarke.[133] Fish, which are taken for human consumption, have been monitored extensively.[33,54,158,163,166] The mono- and di-ortho congeners present were the PCBs 105, 118, 128, 138, 153, 156, 157, 170 and 180. Five of the six major congeners in Wadden Sea fish and Atlantic fish[33] were mono- and di-ortho substituted PCBs. Individual PCB congeners have been determined in fish from the Antarctic.[167] Although the authors only presented congener patterns and did not report which congeners were determined, the overall patterns given in this study resemble those in other studies, underlining that even in remote areas and, hence, after prolonged periods of environmental conditioning, the same PCB patterns are found. The planar congener 77 has been found in fish from the Hudson river[168] and Lake Michigan.[169] Tanabe *et al.*[25] have reported the presence of the three planar congeners in striped mullet (*Mugil cephalus*) at levels of between 2 and 2000 $pg \cdot g^{-1}$, while the total PCB level amounted to 1.2 $\mu g \cdot g^{-1}$.

Except for PCBs 123, 126, 166, 168, 190, all planar, mono- and di-ortho substituted congeners were found in benthic invertebrates.[66,161,164] Wood *et al.*[170] studied the desorption from sediment and bio-uptake for, amongst others, nine of the mono-ortho and di-ortho PCBs by the dipteran larvae, *Chironomus tentans Fabricus*, and found bioconcentration factors ranging from 200 (PCB 118) to 4700 (PCB 189).

The data available on the concentrations of individual PCBs in marine mammals are significantly more detailed than for most other aquatic biota, primarily because of the higher levels found in these species due to the biomagnification through the food chain to the top predators. Table 11 presents the planar, mono- and di-ortho substituted congeners found in marine mammals. It is obvious from Table 11, however, that even for highly contaminated marine mammals, insufficient information is available to allow for an ecotoxicological evaluation, since data are lacking for several of the planar and mono- and di-ortho congeners.

Contradictory findings appear from the reviewed literature with regard to

Table 11 Planar, mono-ortho and di-ortho PCBs reported in marine mammals[a]

Species	*77* ($pg \cdot g^{-1}$)	*126* ($pg \cdot g^{-1}$)	*169* ($pg \cdot g^{-1}$)	*105*	*118*	*128*	*138*	*156*	*157*	*170*	*189*	*ΣPCB* ($\mu g \cdot g^{-1}$)	*Ref.*
Beluga whales *Delphinapterus leucas*					+	+	+			+		66	171
Commerson dolphin *Cephalorynghus commersonii*					+	+	+			+	+	1.5	172
Harbour seal *Phoca vitulina*				+	+		+	+	+	+		–	33
Porpoise *Phocoena phocoena*					+		+			+		–	173
Dalls porpoise (m)	3100	200	150									12	25
Phocoenoides dalli (f)	930	92	53									1.5	
Baird's beaked whale (m)	1600	480	310									2.6	25
Berardius bairdii (f)	700	100	93									1.8	
Finless porpoise *Neophocoena phocoenoides*	14000	890	640									40	25
Pacific white-sided dolphin *Lagenorhyncus abiquidens*	27000	3800	1200									53	25
Killer whale *Orcinus orca*	42000	4000	3600									160	25

[a]Only MC- or mixed-type inducers (cf. Section 2 and Table 5) given; congeners 81, 114, 123, 158, 166, 167 and 168 not reported in any of the marine mammals in this table; +, present (no concentrations given in reference); –, not reported.

congener-specific metabolism or accumulation (see, e.g., refs. 146, 171, 174). This issue has been investigated in more detail by Boon *et al.*[33] in harbour seals from the Dutch Wadden Sea. Congeners containing vicinal hydrogen atoms, i.e., hydrogen atoms bound to adjacent carbon atoms of an aromatic ring, at meta-para positions are always metabolised in the seal body. With vicinal hydrogen atoms at ortho-meta positions, steric hindrance of the ortho hydrogen by an opposing ortho chlorine (as in di-ortho substituted congeners, e.g. PCB 128) may prevent enzymatic breakdown, whereas congeners with mono-ortho substitution (e.g., PCBs 105 and 118) are more easily metabolised.

The decline in the number of otters (*Lutra lutra*) in northwestern Europe has been linked to the PCB levels of adipose tissue of otters found dead.[175-177] The following mono- and di-ortho congeners were found in dead otters in the Netherlands: PCB 105, 118, 128, 138, 153, 156, 167, 170, 180, and 189, with levels ranging between 0.5 and 65 $\mu g \cdot g^{-1}$ (lipid based).[176]

Birds feeding on aquatic organisms sometimes show very high levels of organochlorines (see, e.g., refs. 178–180). Unfortunately, only few of the reports on PCB levels in birds have been addressed to the analysis of individual congeners. In waders from the Dutch Wadden Sea, some 50 congeners were identified.[161] In eggs from eight species of Mediterranean water birds,[53] the highest PCB levels were found in species feeding on fish. Most of the residues contained the 2,4,5-pattern in at least one of the rings of the biphenyl molecule. In eggs of Dutch cormorants, six congeners were determined, including the di-ortho substituted component 138. For the latter congener, concentrations ranged from 1 to 14 $\mu g \cdot g^{-1}$ (total weight).[181] In Adelie penguins from Antarctica some 60 congeners were identified.[146] In all these bird studies the following mono- and di-ortho PCBs were found: PCBs 118, 138, 156 and 170; occasionally also PCBs 105 and 128 were present. In wintering ducks from the Detroit river, with the exception of the three planar PCBs and PCBs 166, 167 and 168, all toxicologically potent PCBs were present.[66]

4.4 Terrestrial Ecosystems

In comparison to aquatic ecosystems, the occurrence and distribution of PCB congeners in terrestrial ecosystems has had significantly less attention. The presence of PCBs in terrestrial ecosystems, particularly in remote areas, is often a result of airborne deposition. In agricultural areas, however, spreading of compost or sewage sludge is a likely source of PCB contamination, and in urban areas there is a direct risk of spillage or seepage from land-fill sites.

There is only one study providing detailed information on individual congeners. The authors report the presence of the mono- and di-ortho congeners 118, 138 and 167 in earthworms.[182] High levels of PCBs have been found in raptors like falcons and sparrow hawks (see, e.g., ref. 183). No information on individual congener content is available, however.

It is known that the amount of chlorination affects the mobility of PCB within plants. Lower chlorinated PCBs are more mobile in soil and may therefore be more readily transported and available for plant uptake.[184] Information on the individual congener concentrations has not been found in the reviewed literature.

4.5 Environmental Monitoring

Due to the relatively low concentrations of the planar congeners and the analytical difficulties accompanying their determination, the presently available amount of data regarding the occurrence of the planar PCBs in the environment is insufficient for a complete understanding of their ecotoxicological significance. In many of the papers discussed above, no attempt was made to identify and quantify the planar PCBs, although their presence in the pertinent samples is far from unlikely. The reasons for not determining the planar congeners have been, apart from those mentioned earlier, the absence of proper standards and the analyst's unawareness of their very presence in the samples. As for several of the mono- and di-ortho PCBs (e.g., PCBs 114 and 123) the same is likely to be true.

Figure 5 depicts the patterns of the planar, mono- and di-ortho substituted PCBs in seston, sediments and various organisms expressed as their relative abundance compared to that of PCB 153. It appears that the mutual ratio of the four prevailing congeners, i.e., PCBs 153, 138, 180 and 170, is more or less constant in all samples. For several of the other congeners preferential accumulation can be seen (e.g., PCBs 105, 118, 123 and 156). For e.g., PCBs 77, 81, 114, 126, 166, 168, 169 and 190 data are, however, usually simply lacking, which underlines the need for additional studies.

Considering the lack of data, it is rather alarming that at the same time some authors[26,58,59,185,186] conclude that the apparent toxic potency of PCB residues exceeds that of the PCDFs and PCDDs and that higher animals as well as humans may be at risk. In particular the levels of the mono-ortho congener 105 and the planar congener 126, and possibly also the mono-ortho congeners 118 and 156 (cf. Table 5), appear to contribute substantially to the total "toxic equivalency factor" of PCB residues.[26,58,59] It is worth noting that the reproductive failure found in harbour seals[32] and in mink[177,187] has been linked to effects of PCBs. Significant toxic equivalent concentrations have not only been found in relatively polluted areas such as northwestern Europe, but also in remote areas. Therefore, extensive monitoring of the planar and some mono- and di-ortho congeners is required for a proper assessment of the impact of the presence of PCBs in (aquatic) wildlife and man. It is precisely in northwestern Europe that symptoms related to PCB contamination have already occurred. The decline in numbers and even regional extinction of certain mammals (e.g., otter[175] and porpoise[188]) in polluted areas in northwestern Europe and its probable link with PCB-induced reproductive failure grimly illustrate the necessity of such monitoring programs.

4.6 Occurrence in Humans

Considerable attention has been paid to the analysis of PCB residues in human tissue. The congeners found in humans will normally reflect the composition of the dietary intake, e.g. fish, animal fat and/or dairy products.[4] The highest levels are found in fat-containing tissues, like adipose tissue and bone marrow, and in human milk. Serum levels of the general population are usually below $15\,ng \cdot g^{-1}$.[189]

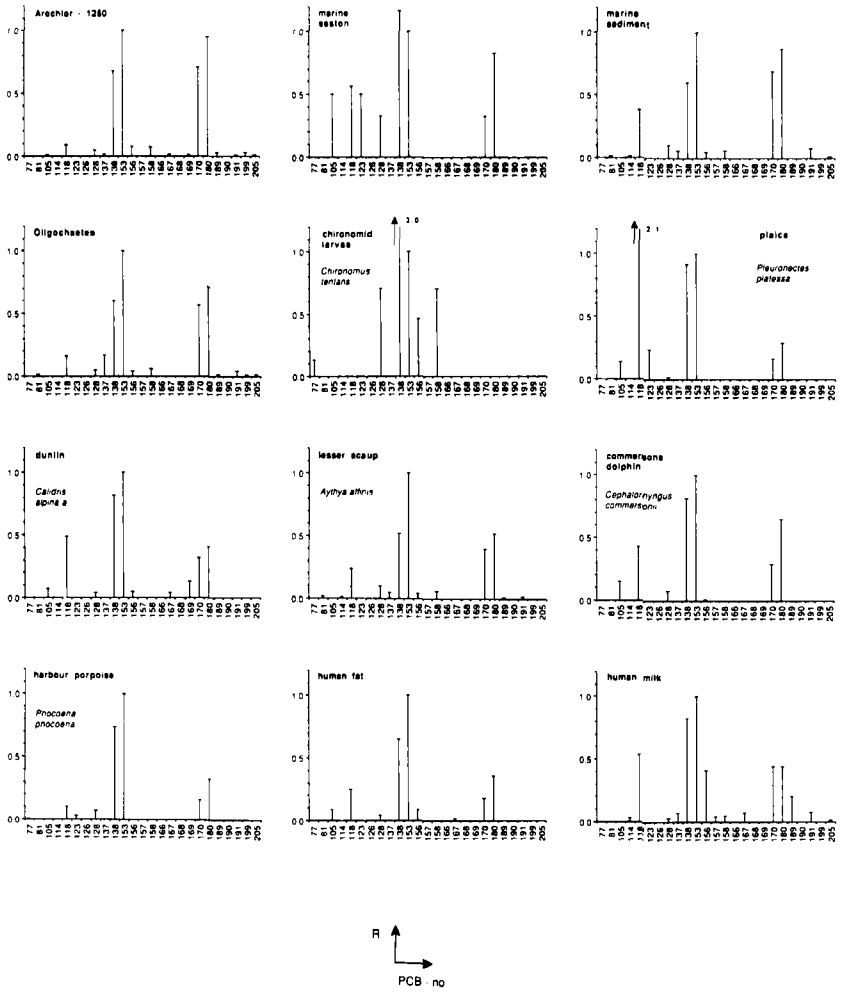

Figure 5 Relative abundance, R, of planar, mono- and di-ortho PCBs in different environmental compartments. Abundance of each congener is expressed as the ratio of its concentration to that of PCB 153.

In human fat from normal and occupationally exposed persons, and in bone-marrow, the following mono- and di-ortho congeners have been identified: PCBs 105, 118, 128, 138, 153, 156, 167, 170, 180 and 189.[4,190–192] In occupationally exposed workers, the PCBs 138 and 153 together account for 36% of the total PCB residue in blood and adipose tissue. This suggests preferential bioaccumulation for PCB congeners with nominal 4-, 2,4- or 3,4-chlorine substitution on both rings. PCBs with the 2,5-substitution pattern appear to be absent, which would suggest that they are metabolised and excreted from the body. Of the planar PCBs, only PCB 77 has been reported to be present in occupationally exposed workers.

Human milk has been monitored regularly because of the potentially harmful exposure of the newborn. Congener patterns found in human milk differed markedly from commercial mixture patterns.[21,193-196] In addition to those congeners also present in adipose tissue, in human milk PCBs 114, 157 and 158 are found. Compared to the industrial mixtures, human milk is considerably enriched in many of the mono- and di-ortho congeners. This is probably due to the preferential metabolism and subsequent excretion of lower chlorinated congeners and the poor absorption of high chlorinated congeners. Hence, a PCB mixture secreted in human milk is 5–10 times more biologically active (cf. Section 2.3) than the corresponding commercial mixture.[24] No planar congeners, and only three mono- and di-ortho congeners, viz. PCBs 128, 138 and 170, were found among the 17 most frequently found PCBs in maternal and fetal blood.[197]

In Japanese (Yusho) and Taiwanese (Yu Cheng) patients who ingested contaminated rice oils the same planar, mono- and di-ortho substituted PCBs occurred as in the normal population.[198,199]

The persistent congeners in humans have a 2,4,5-substitution in one ring and 4-, 2,4- or 2,3,4- in the other.[4,197,200] With the exception of PCB 77 in occupationally exposed workers, until 1988 none of the planar PCBs had been found in humans, although this may be a result of the analytical difficulties discussed above. As a matter of fact, recently the presence of PCBs 77, 126 and 169 was reported in humans, in concentrations higher than those of PCDDs and PCDFs.[26,28] As for the mono- and di-ortho congeners, only two, viz. PCBs 123 and 166, have not been reported in humans, whereas all others have been found in human tissues. Based on their concentrations and relative toxicities, PCBs 105 and 118 are likely to contribute to a major extent to the overall hazardousness of PCBs in humans.

5. DISCUSSION AND CONCLUSIONS

"Polychlorinated biphenyls" (PCBs) is a common name for a seemingly homogeneous group of chlorinated organic compounds which, actually, differ significantly in biological, analytical and ecotoxicological properties. Almost all those differences can be qualitatively, and even quantitatively, explained by the number and positions of the chlorine atoms on the biphenyl skeleton. Non-ortho substituted, so-called planar congeners, with both para and at least two meta positions occupied by chlorine atoms are highly bioactive and toxicologically potent. This activity is also present, albeit somewhat reduced, in the mono- and some of the di-ortho substituted congeners.

Until now, most of the biochemical and toxicological information is only available for terrestrial mammalian animals. Additional information is needed to establish more quantitatively which structural properties govern uptake, metabolism and persistency in the environment. The presence and characterisation of mixed-function oxidase systems in other species, in particular in aquatic biota and in animals at risk, e.g., fish-eating birds (osprey, white-tailed eagle, cormorant), cetaceans (dolphins, porpoises, whales), pinnipeds (seals), mustelids (otter, mink) and humans, in particular newborns, needs further investigation, in order to understand the significance of the levels of PCBs and xenobiotic metabolising

enzymes presently being reported for these organisms (see, e.g., refs. 201, 202). It has to be verified whether structure-activity relationships similar to those in rodents exist for these species; this can possibly be achieved by investigating animals killed in accidents. From such studies, the critical congener levels in the species may then be deduced, which is necessary for establishing environmental standards and further development of environmental policies. In this respect, the additivity of congener toxicity or inducing activity, which is the basis of the toxic equivalence concept, needs careful examination.

The role of PCB metabolites has not been a subject of this review. Yet, since the presence of hydroxy and methylsulphonyl PCBs in biotic samples has been established (see, e.g. ref. 203), these compounds certainly need scientific attention in the years to come. Their role in the biochemical activity and toxicity of PCBs remains unclear until now (see, e.g., ref. 204), and has to be elucidated to validate the present knowledge about the biochemical activity of PCBs. The determination of these metabolites is still in its infancy, however, and therefore requires attention in the near future.

The structural features of PCB congeners, which emanate from the vast amount of scientific literature on this subject, allow for structure-activity relationships. Thus, molecular structure can be used to predict biological activity, toxicity, retention behaviour, identification and environmental occurrence of specific PCB congeners, provided appropriate structural descriptors are available. Further research can make use of achievements from structure-activity relationship studies, e.g., the use of quantum mechanics and molecular modelling data (see, e.g., refs. 45, 47, 205), to improve existing descriptors.

The concentrations of, especially, the planar PCBs in commercial mixtures of PCB are very low. Consequently, their environmental levels are also relatively low and their determination therefore requires large sample sizes. For the toxicologically potent mono- and di-ortho substituted PCBs the levels in environmental samples are, in general, much higher, so that obtaining a sufficiently low LOD is not the main analytical challenge for these compounds (see below). Until recently, planar congeners have not been identified in environmental samples and in humans, more likely as a result of the very low concentrations and the analyst's unawareness of their possible presence, than because of their actual absence. Hence, techniques must be improved to lower the detection limits in GC-ECD and GC-MS with negative chemical ionisation, especially for the planar PCBs. Novel preconcentration and injection techniques, such as, e.g., on-line LC-GC or the use of the PTV injector, already offer good perspectives here. These techniques have to be further improved to cope with the problem of the large sample sizes which are required for the determination of planar PCBs.

High-resolution capillary gas chromatography allows the identification and quantitation of individual PCB congeners. It is now commonly accepted that in environmental samples HRGC is a prerequisite for proper individual congener quantitation, since quantitation on the basis of "total PCB" pattern comparison will be biased due to the congener-specific changes in the total PCB pattern as

compared with the commercial mixtures. In addition, the biochemical and toxicological findings indicate, that the levels of, especially, the planar and mono- and di-ortho PCBs in the environment need to be assessed. For the determination of the planar congeners, additional sample pretreatment steps are required. The same property that appears to govern biochemical activity, i.e. planarity, can be used for the isolation or separation of planar congeners from non-planar ones by carbon chromatography, pyrenyl column HPLC or liquid crystalline capillary column coatings. Two main possibilities have been developed recently: (1) an additional pretreatment, based on carbon adsorption chromatography and (2) multidimensional GC, based on the use of two or more capillary columns. These developments have enabled the detection of planar congeners in a variety of environmental biota, including humans. However, the determination of planar congeners is still very laborious and expensive. Further improvement of both developments is required to reduce both time and costs. As for the mono- and di-ortho substituted congeners, the major analytical issue appears to be their resolution. Although their unambiguous separation on one capillary GC column has been reported at least once,[3] most GC systems employing one capillary column will most probably not be able to resolve all pertinent PCBs satisfactorily. MDGC provides an already existing solution to this problem, and future developments in GC-MS or MS-MS may well offer alternatives. The accuracy of the determination of planar and mono- and di-ortho substituted congeners needs to be established by intercomparison programs and reference materials should be made available.

The number and position of the chlorine atoms in the PCB molecules determine their persistency. Lower chlorinated congeners and congeners having vicinal hydrogen atoms are relatively readily degraded or metabolised in the environment. Thus, different environmental compartments exhibit different congener patterns. The farther removed, whether in time or space, a compartment is from the source of PCBs, the more the PCB pattern will differ from the original commercial mixture pattern. When going from water samples via sediments, invertebrates and fish to fish-eating birds, mammals and humans a general shift from lower chlorinated to higher chlorinated congeners is observed in the PCB patterns. Selective accumulation of at least some of the planar, mono- and di-ortho congeners seems likely, but needs to be further investigated.

As far as can be deduced from the information currently available on PCB-congener-inducing activity and toxicity, and on the occurrence of PCBs in environmental samples, in human tissue and human milk, the mono-ortho pentachlorobiphenyl 105, the planar pentachlorobiphenyl 126 and possibly also the mono-ortho pentachlorobiphenyl 118 and the mono-ortho hexachlorobiphenyl 156 (cf. Figure 6) are the PCBs which present the main risk to the environment in terms of toxic equivalence factors—presumably an even higher risk than the chlorinated dioxins of dibenzofurans. Therefore, in all further monitoring programs trying to assess the PCB burden in view of possible risks for ecosystems or man, these four congeners (Figure 6) should invariably be included.

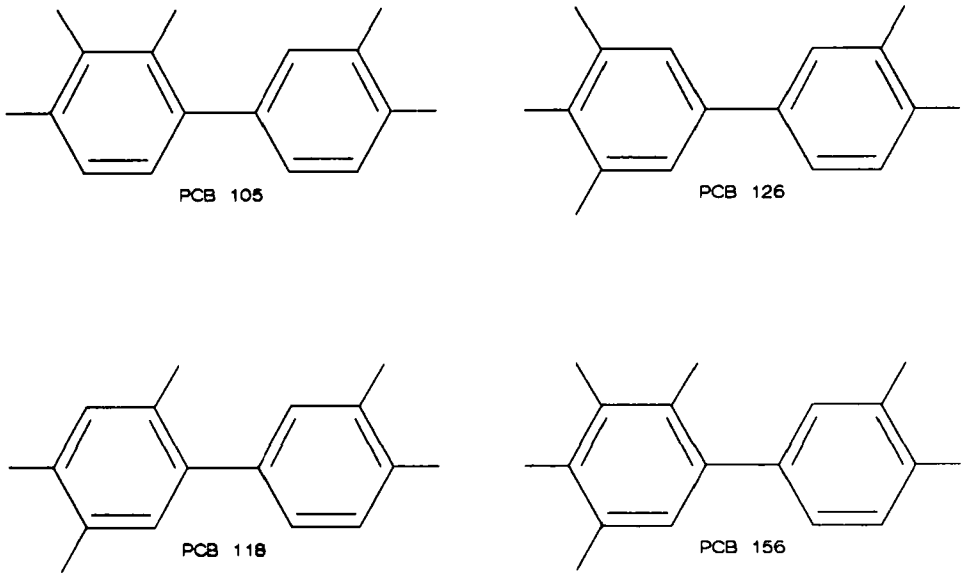

Figure 6 Structures of four PCBs recommended for incorporation in monitoring programs.

List of Abbreviations

Ah	Aryl hydrocarbon (receptor)
AHH	Aryl hydrocarbon hydroxylase
CB	Chlorinated biphenyl
CRM	Certified reference material
EC_{50}	Concentration necessary to reduce specific binding to 50% of maximum value or to produce 50% of maximum enzyme induction
ECD	Electron capture detection
ED_{50}	Dose necessary to produce 50% of maximum enzyme induction
EROD	Ethoxy resorufin-O-deethylase
GC	Gas chromatography
GPC	Gel permeation chromatography
HPLC	High-performance liquid chromatography
HRGC	High-resolution capillary gas chromatography
LC	Liquid chromatography
LC-GC	Liquid chromatography with on-line gas chromatography
LOD	Limit of detection
MC	3-Methylcholanthrene
MDGC	Multidimensional gas chromatography
MFO	Mixed-function oxidase
MID	Multiple ion detection
MS	Mass spectrometry
PB	Phenobarbital
PCB	Polychlorinated biphenyl

PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzofuran
PGC	Porous graphite carbon
PTV	Programmed temperature vaporiser
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TCDF	2,3,7,8-Tetrachlorodibenzofuran
TEC	TCDD toxic equivalence concentration
TEF	Toxic equivalence factor

Acknowledgement

The authors wish to thank Bert van Hattum for the fruitful discussions on the significance of PCB contamination.

References

1. K. Ballschmiter and M. Zell, *Fres. Z. Anal. Chem.* **302,** 20 (1980).
2. P. de Voogt and U. A. Th. Brinkman, *Halogenated Biphenyls and Related Products* (2nd ed.) (R. D. Kimbrough and A. A. Jensen, eds.) (Elsevier, Amsterdam, The Netherlands, 1989), pp. 1–45.
3. M. D. Mullin, C. M. Pochini, S. McCrindle, M. Romkes, S. H. Safe and L. M. Safe, *Environ. Sci. Technol.* **18,** 468 (1984).
4. K. C. Jones, *Sci. Total Environ.* **68,** 141 (1988).
5. P. W. Albro, K. Haseman, T. A. Clemmer, and B. J. Corbett, *J. Chromatogr.* **136,** 147 (1977).
6. P. W. Albro and C. E. Parker, *J. Chromatogr.* **169,** 161 (1979).
7. P. W. Albro, J. T. Corbett and J. L. Schroeder, *J. Chromatogr.* **205,** 103 (1981).
8. J. C. Duinker, M. T. J. Hillebrand and J. P. Boon, *Neth. J. Sea Res.* **17,** 19 (1983).
9. P. Lišhák, *Chlorácia Bifenylu* (technical report), pp. 62–63, Chemko, n.p. Strážske, CSSR. (1981), (in Czech.).
10. N. Kannan, S. Tanabe, T. Wakimoto and R. Tatsukawa, *J. Assoc. Off. Anal. Chem.* **70,** 451 (1987).
11. J. F. Schneider, G. T. Reedy and D. G. Ettinger, *J. Chromatogr. Sci.* **23,** 49 (1985).
12. M. D. Erickson, *Analytical Chemistry of PCBs* (Ann Arbor Science, Ann Arbor, MI, USA, 1986).
13. S. Tanabe, N. Kannan, A. Subramanian, S. Watanabe, M. Ono and R. Tatsukawa, *Chemosphere* **16,** 1965 (1987).
14. S. Tanabe, *Environ. Pollut.* **50,** 5 (1988).
15. R. D. Kimbrough (ed.) *Halogenated Biphenyls Terphenyls, Naphthalenes, Dibenzodioxins and Related Products* (Elsevier, Amsterdam, The Netherlands, 1980).
16. J. S. Waid (ed.) *PCBs and the Environment* (CRC Press, Boca Raton, FL, USA, 1986).
17. R. D. Kimbrough and A. A. Jensen (eds.) *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products* (2nd ed.) (Elsevier, Amsterdam, The Netherlands, 1989).
18. A. De Kok, R. B. Geerdink, R. W. Frei and U. A. Th. Brinkman, *Intern. J. Environ. Anal. Chem.* **9,** 301 (1981).
19. A. De Kok, R. B. Geerdink, R. W. Frei and U. A. Th. Brinkman, *Intern. J. Environ. Anal. Chem.* **11,** 17 (1982).
20. S. Jensen, *New Scientist* **32,** 612 (1966).
21. S. Safe, L. Safe and M. Mullin, *PCBs, Environmental Occurrence and Analysis* (S. Safe, ed.) (Springer, Berlin, FRG, 1987), pp. 1–13.
22. A. Parkinson, S. H. Safe, L. W. Robertson, P. E. Thomas, D. E. Ryan, L. M. Reik and W. Levin, *J. Biol. Chem.* **258,** 5967 (1983).
23. S. Safe, S. Bandiera, T. Sawyer, L. Robertson, L. Safe, A. Parkinson, P. E. Thomas, D. E. Ryan, L. M. Reik, W. Levin, M. A. Denomme and T. Fujita, *Environ. Health Perspect.* **60,** 47 (1985).
24. A. Parkinson and S. Safe, *Mammalian Biologic and Toxic Effects of PCBs* (S. Safe, ed.) (Springer, Heidelberg, FRG, 1987), pp. 49–75.
25. S. Tanabe, N. Kannan, A. Subramanian, S. Watanabe and R. Tatsukawa, *Environ. Pollut.* **47,** 147 (1987).

26. S. Tanabe, N. Kannan and R. Tatsukawa, *Occurrence, Behaviour and Toxic Implications of Coplanar PCBs in Humans and Wildlife* (Proc. Symp. Transboundary Pollution, Toronto, Canada, 1989), p. 129.
27. N. Kannan, S. Tanabe and R. Tatsukawa, *Bull. Environ. Contam. Toxicol.* **41,** 267 (1988).
28. N. Kannan, S. Tanabe and R. Tatsukawa, *Arch. Environ. Health.* **43,** 11 (1988).
29. E. E. McConnell, *Acute and Chronic Toxicity, Carcinogenesis, Reproduction, Teratogenesis and Mutagenesis in Animals* (R. D. Kimbrough, ed.) (Elsevier, Amsterdam, The Netherlands, 1980), pp. 109–150.
30. A. Bergman and M. Olsson, *Finnish Game Res.* **44,** 47 (1986).
31. P. J. H. Reijnders, *Neth. J. Sea Res.* **14,** 30 (1980).
32. P. J. H. Reijnders, *Nature* **324,** 456 (1987).
33. J. P. Boon, P. J. H. Reijnders, J. Dols, P. Wensvoort and M. T. J. Hillebrand, *Aquat. Toxicol.* **10,** 307 (1987).
34. A. Brouwer, P. J. H. Reijnders and J. H. Koeman, *Aquat. Toxicol.* **15,** 99 (1989).
35. L. Reutergårdh and A. Knap, *Contaminants and Marine Mammals: A Review.* Report of the ICES Advisory Committee on Marine Pollution. ICES Cooperative Research Report 150, p. 36. ICES, Copenhagen, Denmark, 1987.
36. J. U. Clarke, *Chemosphere* **15,** 275 (1986).
37. M. A. Denomme, B. Leece, A. Li. Towner and S. Safe, *Biochem. Pharmacol.* **35,** 277 (1986).
38. J. A. Goldstein and S. Safe, *Mechanism of Action and Structure-Activity Relationships for the Chlorinated Dibenzo-p-Dioxins and Related Compounds* (R. D. Kimbrough and A. A. Jensen, eds.) (Elsevier, Amsterdam, The Netherlands, 1989), pp. 239–293.
39. J. D. McKinney, K. Chae, E. E. McConnell and L. S. Birnbaum, *Environ. Health Perspect.* **60,** 57 (1985).
40. S. S. Thorgeirsson and D. W. Nebert, *Adv. Cancer Res.* **25,** 149 (1977).
41. J. D. McKinney and P. Singh, *Chem.-Biol. Interact.* **33,** 271 (1981).
42. L. Van Bree, F. A. M. Redegeld and J. De Vries, *Toxicology* **42,** 259 (1986).
43. S. Safe, S. Bandiera, T. Sawyer, B. Zmudzka, G. Mason, M. Romkes, M. A. Denomme, J. Sparling, A. B. Okey and T. Fujita, *Environ. Health Perspect.* **61,** 21 (1985).
44. S. Sassa, O. Sugita, N. Ohnuma, S. Imajo, T. Okumura, T. Noguchi and A. Kappas, *Biochem. J.* **235,** 291 (1986).
45. J. D. McKinney, K. E. Gottschalk and L. Pedersen, *J. Molec. Struct.* **104,** 445 (1983).
46. J. D. McKinney, G. A. Long and L. Pedersen, *Quant. Struct.-Act. Relat.* **3,** 99 (1984).
47. J. D. McKinney, T. Darden, M. A. Lyerly and L. G. Pedersen, *Quant. Struct.-Act. Relat.* **4,** 166 (1985).
48. S. Bandiera, T. W. Sawyer, M. A. Campbell, S. Fujita and S. Safe, *Biochem. Biopharmacol.* **32,** 3803 (1983).
49. A. Poland and E. Glover, *Mol. Pharmacol.* **13,** 924 (1977).
50. J. A. Bradlaw and J. L. Casterline, *J. Assoc. Off. Anal. Chem.* **62,** 904 (1979).
51. S. Safe, *Chemosphere* **16,** 791 (1987).
52. T. Sawyer and S. Safe, *Toxicol. Lett.* **13,** 87 (1982).
53. S. Focardi, C. Leonzio and C. Fossi, *Environ. Pollut.* **52,** 243 (1988).
54. J. de Boer, *Chemosphere* **17,** 1811 (1988).
55. D. E. Wells, J. de Boer, L. G. M. Th. Tuinstra, L. Reutergårdh and B. Griepink, *Fres. Z. Anal. Chem.* **332,** 591 (1988).
56. F. O. Dorgelo, *Estimation of the Toxicity of a Number of PCDDs, PCDFs and PCBs in Dutch Eel Compared With That of Other Components of These Groups of Chemicals.* RIKILT, report 88.49, Wageningen, The Netherlands (in Dutch, English summary).
57. J. N. Huckins, T. D. Schwartz, J. D. Petty and L. M. Smith, *Chemosphere* **17,** 1995 (1988).
58. R. J. Norstrom, M. Simon, M. J. Mulvihill and D. C. G. Muir, *Toxic Non-ortho and Mono-ortho PCBs in Canadian Wildlife—Levels and Possible Significance* (Proc. Symp. Transboundary Pollution, Toronto, Canada, 1989), p. 129.
59. L. M. Smith, T. R. Schwartz, K. Feltz and T. J. Kubiak, *Determination and Significance in Environmental Samples of the Most Toxic PCB Congeners* (Proc. Symp. Transboundary Pollution, Toronto, Canada, 1989), p. 128.

60. J. M. Haake, S. Safe, K. Mayura and T. D. Phillips, *Toxicol. Lett.* **38,** 229 (1987).
61. C. Haux and L. Forlin, *Ambio* **17,** 376 (1988).
62. M. Celander and L. Forlin, *Immunochemical Quantitation of βNF-inducible Cytochrome P450 in Rainbow Trout and Perch* (Cytochrome P450, Biochemistry and Biophysics. I. Schuster, ed.) (Taylor and Francis, Basingstoke, UK, 1988).
63. K. H. Palmork, J. C. Duinker, A. Knap and J. P. Villeneuve, *Organochlorines* (Scientific Report of the Intercalibration Exercise. Intergovernmental Oceanographic Commission Technical Series. Report No. 22 UNESCO, Paris) (ISBN 92-3-102077-3), (1982), pp. 60–83.
64. WHO—World Health Organisation, *Environ. Health Crit.* **2,** (Geneva, WHO, 1976), p. 85.
65. L. Reutergårdh, *Identification and Distribution of Chlorinated Organic Pollutants in the Environment.* Thesis (Stockholm University, Stockholm, Sweden, 1988).
66. V. E. Smith, J. M. Spurr, J. C. Filkins and J. D. Jones, *J. Great Lakes Res.* **11,** 231 (1985).
67. D. J. H. Phillips, *Quantitative Aquatic Biological Indicators: Their Use to Monitor Trace Metal and Organochlorine Pollution* (London Applied Science Ltd., UK, 1980).
68. J. V. Hinshaw, *LC-GC Intern.* **2**(2), 24 (1989).
69. E. Noorozian, F. A. Maris, M. W. F. Nielen, R. R. Frei, G. J. de Jong and U. A. Th. Brinkman, *J. High Res. Chromatogr. Chromatogr. Commun.* **10,** 17 (1987).
70. F. A. Maris, E. Noorozian, R. R. Otten, R. C. J. M. van Dijck, G. J. de Jong and U. A. Th. Brinkman, *J. High Resol. Chromatogr. Chromatogr. Commun.* **11,** 197 (1988).
71. J. D. Miller, R. E. Thomas and H. J. Schattenberg, *Anal. Chem.* **53,** 214 (1981).
72. J. D. Millar, R. E. Thomas and D. E. Johnson, *Determination of Pesticides and PCBs in Industrial and Municipal Waste Waters,* US EPA contract 68-03-2606. Rpt. No. EPA-600/4-82-023 (NTIS PB82-214222).
73. J. J. Delfino and D. B. Easty, *Anal. Chem.* **51,** 2235 (1979).
74. M. Godefroot, M. Stechele, P. Sandra and M. Verzele, *J. High. Res. Chromatogr. Chromatogr. Commun.* **5,** 75 (1984).
75. G. B. Nickerson and S. T. Likens, *J. Chromatogr.* **21,** 1 (1966).
76. J. A. Coburn, I. A. Valdmanis and A. S. Y. Chau, *J. Assoc. Off. Anal. Chem.* **60,** 224 (1977).
77. D. E. Wells and S. J. Johnstone, *Water, Air, Soil Pollut.* **9,** 271 (1978).
78. P. R. Musty and G. Nickless, *J. Chromatogr.* **89,** 185 (1974).
79. N. Picer and M. Picer, *J. Chromatogr.* **193,** 357 (1980).
80. D. S. Gallis, A. Defner-Hallowel, J. C. White and J. Waber, *Toxicol.* **31,** 285 (1983).
81. C. F. Rodriguez, W. A. McMahon and R. E. Thomas, *Method Development for the Determination of Polychlorinated Hydrocarbons in Municipal Sludge.* Final report, Contract 68-03-2606, EPA-600/2-80-290, NTIS PB 82-234 071.
82. D. F. Goerlitz and L. M. Law, *J. Assoc. Off. Anal. Chem.* **57,** 176 (1974).
83. G. Eder, *Chemosphere* **5,** 101 (1976).
84. S. Jensen, L. Renberg and L. Reutergårdh, *Anal. Chem.* **49,** 316 (1977).
85. A. S. Y. Chau and L. J. Babjak, *J. Assoc. Off. Anmal. Chem.* **62,** 107 (1979).
86. B. Bush, L. A. Shane, M. Wahler and M. P. Brown, *Chemosphere* **16,** 733 (1987).
87. G. Seidl and K. Ballschmitter, *Chemosphere* **5,** 373 (1976).
88. T. A. Bellar and J. J. Lichtenberg, *Some Factors Affecting the Recovery of Polychlorinated Biphenyls (PCBs) from Water and Bottom Samples* (Special Techniques Publication 573 ASTM, Philadelphia PA, USA, 1975, pp. 206–219 (1975).
89. T. A. Bellar, J. J. Lichtenberg and S. C. Lonneman, *Recovery of Organic Compounds from Environmentally Contaminated Bottom Materials* (R. A. Baker, ed.) (Contaminants and Sediments, 2, Ch. 4, Ann Arbor Science, Ann Arbor, MI, USA, 1980).
90. R. R. Watts (ed.), *Analysis of Pesticide Residues in Human and Environmental Samples, a Compilation of Methods Selected for Use in Pesticide Monitoring Programs.* US Environmental Protection Agency, Research Triangle Park, NC, EPA-600/8-80-038 (June, 1980).
91. L. L. Needham, V. W. Burse and H. A. Price, *J. Assoc. Off. Anal. Chem.* **64,** 1131 (1981).
92. V. W. Burse, L. L. Needham, M. P. Korver, C. R. Lapeza (Jnr), J. A. Liddle and D. D. Bayse, *J. Assoc. Off. Anal. Chem.* **66,** 40 (1983).
93. J. D. McKinney, L. Moore, A. Prokepetz and D. B. Walters, *J. Assoc. Off. Anal. Chem.* **67,** 122 (1984).

94. E. L. Baker, P. J. Landrigan, C. J. Clueck, M. M. Zack (Jnr), J. A. Liddle, V. W. Burse, W. J. Housworth and L. L. Needham, *Amer. J. Epidemiol.* **112** 553 (1980).
95. V. Zitko, *Bull Environ. Contam. Toxicol.* **6,** 464 (1973).
96. J. de Boer, *Chemosphere* **17,** 1803 (1988).
97. D. W. Kuehl, E. N. Leonard, K. J. Welch and G. D. Veith, *J. Assoc. Off. Anal. Chem.* **63,** 1238 (1980).
98. S. D. Kilikdis, J. E. Psomas, A. P. Kamarianos and A. G. Panetsos, *Bull. Environ. Contam. Toxicol.* **26,** 496 (1981).
99. M. L. Hattula, *Bull. Environ. Contam. Toxicol.* **12,** 301 (1974).
100. D. L. Stalling, *Pestic. Chem. Proc., Intl. IUPAC Congr. Pest. Chem., 2nd* **4,** 413 (1971).
101. D. R. Erney, *J. Assoc. Off. Anal. Chem.* **57,** 576 (1974).
102. G. D. Veith and L. M. Kiwus, *Bull. Environ. Contam. Toxicol.* **17,** 631 (1977).
103. R. C. Dougherty, *Toxic Residues and Pollutants* (G. R. Waller and O. C. Dermer, eds.) (Wiley-Interscience Publication, New York, NY, USA, 1980), pp. 951–968.
104. M. G. Castelli, G. P. Martelli, C. Spagone, L. Cappellini and R. Fanelli, *Chemosphere* **12,** 291 (1983).
105. L. G. M. T. Tuinstra, A. H. Roos and G. A. Werdmuller, *J. Assoc. Off. Anal. Chem.* **68,** 756 (1985).
106. F. Van der Valk and Q. T. Dao, *Chemosphere* **17,** 1735 (1988).
107. P. G. Murphy, *J. Assoc. Off. Anal. Chem.* **55,** 1360 (1972).
108. M. L. Hattula, *Bull. Environ. Contam. Toxicol.* **12,** 331 (1974).
109. L. L. Lamparsku, T. J. Nestrick and R. H. Stehl, *Anal. Chem.* **51,** 1453 (1979).
110. L. M. Smith, D. L. Stalling and J. L. Johnson, *Anal. Chem.* **56,** 1830 (1984).
111. H. Buchert, S. Bikler and K. Ballschmiter, *Fres. Z. Anal. Chem.* **313,** 1 (1982).
112. T. S. Bates and R. Carpenter, *Anal. Chem.*, **51,** 551 (1979).
113. P. E. Mattsson and S. Nygren, *J. Chromatogr.* **124,** 265 (1976).
114. D. L. Stalling, L. M. Smith and J. D. Petty, *Approaches to Comprehensive Analysis of Persistent Halogenated Environmental Contaminants* (Measurement of Organic Pollutants in Water and Wastewater. ASTM STP 686. Van Hall, C. E. ed., 1979), pp. 302–323.
115. P. de Voogt, J. C. Klamer and H. Govers, *J. Chromatogr.* **363,** 407 (1986).
116. D. E. Wells, *Pure Appl. Chem.* **60,** 1437 (1988).
117. L. M. Smith and J. L. Johnson, *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (G. Choudhary, L. H. Keith and C. Rappe, eds.) (Ann Arbor Science Publishers, Ann Arbor, MI, USA, 1983), pp. 321–332.
118. L. R. Kamops, W. J. Trotter, J. S. Young, A. C. Smith, J. A. G. Roach and S. W. Page, *Bull. Environ. Contam. Toxicol.* **23,** 51 (1979).
119. J. Teichmann, A. Benevue and J. W. Hylin, *J. Chromatogr.* **151,** 155 (1978).
120. S. Jensen and G. Sundström, *Ambio* **3,** 70 (1974).
121. J. N. Huckins, D. L. Stalling and J. D. Petty, *J. Assoc. Off. Anal. Chem.* **63,** 750 (1980).
122. P. W. O'Keefe, R. M. Smith, D. R. Hilker, K. M. Aldous and W. Gilday, *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (L. H. Keith, C. Rappe and G. Choudhary, eds.) (Butterworth, Boston, MA, USA, 1985), pp. 111–124.
123. G. R. Shaw and D. W. Connell, *Environ. Sci. Technol.* **18,** 18 (1984).
124. P. Schmid, M. E. Suter-Hofmann and C. Schlatter, *Chemosphere* **18,** 1741 (1989).
125. C. S. Creaser and A. Al-Haddad, *Anal. Chem.* **61,** 1300 (1989).
126. J. H. Knox, B. Kaur and G. R. Millward, *J. Chromatogr.* **352,** 3 (1986).
127. L. G. M. T. Tuinstra, J. J. M. Driessen, H. J. Keukens, T. J. van Munsteren, A. H. Roos and W. A. Traag, *Intern. J. Environ. Anal. Chem.* **14,** 147 (1983).
128. DIN 51527, *Determination of Polychlorinated Biphenyls (in Lubricating Oils)* (Beuth Verlag GmbH, Berlin, FRG, 1987).
129. J. W. Wegener, P. de Voogt and H. Govers, *Staatsblad* **145,** 3 (1988) (in Dutch).
130. J. C. Duinker, D. E. Schultz and G. Petrick, *Marine Pollut. Bull.* **19,** 19 (1988).
131. A. H. Roos, P. G. M. Kienhuis, W. A. Traag, and L. G. Th. M. Tuinstra, *Intern. J. Environ. Anal. Chem.* **36,** 155 (1989).
132. P. de Voogt, J. C. Klamer and U. A. Th. Brinkman, *Bull. Environ. Contam. Toxicol.* **32,** 45 (1984).
133. V. A. McFarland and J. U. Clarke, *Environ. Health Perspect.* **81,** 225 (1989).

134. J. C. Duinker, D. E. Schultz and G. Petrick, *Anal. Chem.* **60,** 478 (1988).
135. S. Tanabe, N. Kannan, T. Wakimoto and R. Tatsukawa, *Intern. J. Environ. Anal. Chem.* **29,** 199 (1987).
136. F. R. Guenther, R. N. Chesler and R. E. Rebbert, *The Analysis of PCBs by Multidimensional Gas Chromatography.* Proc. 10th Intern. Symp. on Capillary Chromatography, Riva, Italy (1989), pp. 666–672.
137. W. L. Zielinski, Jr., M. M. Miller, G. Ulma and S. P. Wasik, *Anal. Chem.* **58,** 2692 (1986).
138. A. Sabljic, *J. Chromatogr.* **319,** 1 (1985).
139. L. B. Kier and L. H. Hall, *Molecular Connectivity in Structure-Activity Analysis* (Research Studies Press, Letchworth, U.K., 1986).
140. R. Kaliszan, *Quantitative Structure-Chromatographic Retention Relationships* (Wiley and Sons, New York, NY, USA, 1987).
141. A. Robbat, Jr., G. Xyrafas and D. Marshall, *Anal. Chem.* **60,** 982 (1988).
142. S. Fachetti, *Adv. Mass Spectrom.* **11,** 1542 (1989).
143. F. Artigas, E. Martinez and E. Gelpi, *Biomed. Environ. Mass Spectrom.* **16,** 279 (1988).
144. J. C. Duinker and M. T. J. Hillebrand, *Environ. Sci. Technol.* **17,** 449 (1983).
145. B. Bush, M. J. Murphy, S. Connor, J. Snow and E. Barnard, *J. Chromatogr. Sci.* **23,** 509 (1985).
146. S. Tanabe, A. N. Subramanian, H. Kidaka and R. Tatsukawa, *Chemosphere* **15,** 343 (1986).
147. K. Ballschmiter, W. Schaefer and H. Buchert, *Fres. Z. Anal. Chem.* **326,** 253 (1987).
148. N. Kannan, S. Tanabe, T. Wakimoto and R. Tatsukawa, *Chemosphere* **16,** 1631 (1987).
149. A. L. Alford-Stevens, T. A. Bellar, T. A. Eichelberger, J. W. and W. L. Budde, *Anal. Chem.* **58,** 2014 (1986).
150. T. R. Schwartz, D. L. Stalling and C. L. Rice, *Environ. Sci. Technol.* **21,** 72 (1987).
151. J. P. Boon and J. C. Duinker, *Marine Environ. Res.* **17,** 301 (1985).
152. J. P. Boon and F. Eijgenraam, *Marine Environ. Res.* **24,** 3 (1988).
153. I. G. Sipes and R. G. Schnellmann, *PCBs: Mammalian and Environmental Toxicology* (S. Safe, ed.) (Springer, Berlin, FRG, 1987), pp. 97–110.
154. N. Kannan, S. Tanabe, R. Tatsukawa and D. J. H. Phillips, *Environ. Pollut.* **56,** 65 (1989).
155. J. Paasivirta, J. Koistinen, E. Mannilo, J. Tarhanen, P. J. Vuorinen and N. Kannan, *Coplanar PCBs in Finnish Wildlife* (Proc. Symp. Transboundary Pollution, Toronto, Canada, 1989), p. 129.
156. A. Opperhuizen, A. P. C. F. Gobas, J. M. D. van der Steen and O. Hutzinger, *Environ. Sci. Technol.* **22,** 638 (1988).
157. H. Aiking, *Micropollutants in Organisms, Sediments and Particular Matter (MIVEOS): Interpretation of Particulate Matter Data.* Institute for Environmental Studies, E-87/7, Free University, Amsterdam, The Netherlands (1987) (in Dutch).
158. J. P. Boon and J. C. Duinker, *Aquat. Toxicol.* **7,** 119 (1985).
159. B. Bush, K. W. Simpson, L. Shane and R. R. Koblintz, *Bull. Environ. Contam. Toxicol.* **34,** 96 (1985).
160. J. P. Boon, M. B. van Zantvoort, M. J. M. A. Govaert and J. C. Duinker, *Neth. J. Sea Res.* **19,** 93 (1985).
161. P. de Voogt, J. C. Klamer, A. A. Goede and H. Govers, *Accumulation of Organochlorine Compounds in Waders From the Dutch Wadden Sea.* Free University (IVM R-85/7), Amsterdam, The Netherlands (1985).
162. C. Strang, S. P. Levine, B. P. Orlan, T. S. Gouda and W. A. Saner, *J. Chromatogr.* **314,** 482 (1984).
163. M. Zell and K. Ballschmiter, *Fres. Z. Anal. Chem.* **304,** 337 (1980).
164. J. P. Boon, Thesis (University of Groningen, The Netherlands, 1986).
165. A. J. Niimi and B. G. Oliver, *Can. J. Fish. Aquat. Sci.* **40,** 1388 (1983).
166. D. Rossell, P. Honsberger and J. Taradellas, *Intern. J. Environ. Anal. Chem.* **31,** 219 (1987).
167. B. R. Subramanian, S. Tanabe, H. Kidaka and R. Tatsukawa, *Arch. Environ. Contam. Toxicol.* **12,** 621 (1983).
168. D. L. Stalling, J. N. Huckins and J. D. Petty, *Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment* (B. K. Afgahn and D. Mackay, eds.) (Plenum Press, New York, NY, USA, 1980), pp. 131–139.
169. N. J. Trotter, S. J. V. Young, J. L. Casterline, J. A. Bradshaw and L. R. Kamps, *J. Assoc. Offic. Anal. Chem.* **65,** 838 (1982).

170. L. W. Wood, G. Y. Rhee, B. Bush and E. Barnard, *Water Res.* **21,** 875 (1987).
171. R. Massé, D. Martineau, L. Tremblay and P. Beland, *Arch. Environ. Contam. Toxicol.* **15,** 567 (1986).
172. A. Abarnou, D. Robineau and P. Michel, *Oceanol. Acta* **9,** 19 (1986).
173. J. C. Duinker and M. T. J. Hillebrand, *Bull. Environ. Contam. Toxicol.* **31,** 25 (1983).
174. S. Tanabe, R. Tatsukawa, K. Markuyama and N. Miyazaki, *Agric. Biol. Chem.* **46,** 1249 (1982).
175. C. F. Mason and S. M. Macdonald, *Otters: Ecology and Conservation* (Cambridge University Press, Cambridge, U.K. 1986).
176. S. Broekhuizen and E. M. de Ruiter-Dijkman, *Lutra* **31,** 68 (1988) (in Dutch, English summary).
177. S. Jensen, J. E. Kihlström, M. Olsson, C. Lundberg and J. Örberg, *Ambio* **6,** 239 (1977).
178. B. Helander, M. Olsson and L. Reutergårdh, *Holarct. Ecol.* **5,** 349 (1982).
179. D. B. Peakall, *PCBs and the Environment* (J. S. Waid, ed.) (CRC Press, Boca Raton, FL, USA, 1986), Vol. 2, pp. 31–47.
180. S. N. Wiemeyer, C. M. Bunck and A. J. Krynitsky, *Arch. Environ. Contam. Toxicol.* **17,** 767 (1988).
181. M. Van Schaick and P. de Voogt, *Concentrations of PCB Congeners in Eggs and Regurgitated Pellets of Dutch Cormorants.* (Free University W-89/173, Amsterdam, The Netherlands, 1989) (in Dutch, English summary).
182. J. Taradellas, P. Diercxsens and M. B. Bouche, *Methods of Extraction and Analysis of PCBs from Earthworms,* Proc. 12th Ann. Symp. Anal. Chem. Pollut. (Amsterdam, The Netherlands, 1982), p. 74.
183. I. Prestt, D. J. Jefferies and N. W. Moore, *Environ. Pollut.* **1,** 3 (1970).
184. H. J. Strek and J. B. Weber, *Environ. Pollut.* **28A,** 291 (1982).
185. J. Tarhanen, J. Koistinen, J. Paasivirta, P. J. Vuorinen, J. Koivusaari, I. Nuuja, N. Kannan and T. Tatsukawa, *Chemosphere* **18,** 1067 (1989).
186. S. Tanabe, N. Kannan, M. Ono and R. Tatsukawa, *Chemosphere* **18,** 485 (1989).
187. R. J. Aulerich, S. J. Bursioan, W. J. Breslin, B. A. Olson and R. K. Ringer, *J. Toxicol. Environ. Health* **15,** 63 (1985).
188. M. Gilbertson, *Halogenated Biphenyls and Related Products* (2nd ed.) (R. D. Kimbrough and A. A. Jensen, eds.) (Elsevier, Amsterdam, The Netherlands, 1989), pp. 103–127.
189. S. Safe, *PCBs: Mammalian and Environmental Toxicology* (S. Safe, ed.) (Springer, Berlin, FRG, 1987), pp. 133–145.
190. S. Focardi, C. Fossi, C. Leonzio and R. Romei, *Bull. Environ. Contam. Toxicol.* **36,** 644 (1986).
191. M. S. Wolf, J. Thornton, A. Fishbein, R. Lilis and I. J. Selikoff, *Toxicol. Appl. Pharmacol.* **62,** 294 (1982).
192. S. Dmochewitz and K. Ballschmiter, *Fres. Z. Anal. Chem.* **310,** 6 (1982).
193. A. Parkinson, L. W. Robertson and S. Safe, *Biochem. Biophys. Res. Comm.* **96,** 882 (1980).
194. B. Bush, J. Snow, S. Connor and R. Koblints, *Arch. Environ. Contam. Toxicol.* **14,** 443 (1985).
195. K.-H. Hahne, W. Heeschen and A. Bluethgen, *Milchwiss,* **41,** 414 (1986).
196. J. Mes and P. Y. Lau, *Bull. Environ. Contam. Toxicol.* **31,** 639 (1983).
197. B. Bush, J. Snow and R. Koblintz, *Arch. Environ. Contam. Toxicol.* **13,** 517 (1984).
198. Y. Masuda, H. Kuroki, K. Haraguchi and J. Nagayama, *Environ. Health Perspect.* **59,** 53 (1985).
199. P. H. Chen and R. A. Hites, *Chemosphere* **12,** 1507 (1983).
200. T. Yakushiji, I. Watanabe, K. Kuwabara, R. Tanaka, R. Kashimoto, N. Kunita and I. Hara, *Arch. Environ. Contam. Toxicol.* **13,** 342 (1984).
201. C. Fossi, C. Leonzio, S. Focardi and A. Renzoni, *Bull. Environ. Contam. Toxicol.* **41,** 365 (1988).
202. B. A. Rattner, D. J. Hoffman and C. M. Marn, *Environ. Toxicol. Chem.* **8,** 1093 (1989).
203. K. Haraguchi, H. Kuroki and Y. Masuda, *Chemosphere* **18,** 477 (1989).
204. L. Van Bree, *Interactions of Dihalogenated Biphenyls with Hepatic Xenobiotic-metabolizing Enzymes* (Ph.D. Thesis, Free University, Amsterdam, The Netherlands, 1987).
205. R. Upton and H. Gasking, *Intern. Lab.* (September, 1988), pp. 24–28.
206. P. Haglund, L. Asplund, H. Järnberg and B. Jansson, *J. Chromatogr.* **507,** 389 (1990).