Physico-chemical characteristics and quantitative structure-activity relationships of PCBs

by

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Akademisk avhandling

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Title Physico-chemical characteristics and quantitative structure-activity relationships of PCBs

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Abstract

The polychlorinated biphenyls (PCBs) comprise a group of 209 congeners varying in the number of chlorine atoms and substitution patterns. These compounds tend to be biomagnified in foodwebs and have been shown to induce an array of effects in exposed organisms. The structural characteristics of the PCBs influence their potency as well as mechanism of action. In order to assess the biological potency of these compounds a multi-step quantitative structure-activity relationship (QSAR) procedure was used in the project described in this thesis.

The ultraviolet absorption (UV) spectra were measured for all 209 PCBs, and digitised for use as physico-chemical descriptors. Interpretations of the spectra using principal component analysis (PCA) showed the number of *ortho* chlorine atoms and *para-para* substitution patterns to be significant. Additional physico-chemical descriptors were derived from semi-empirical calculations. These included various molecular energies, the ionisation potential, electron affinity, dipole moments, and the internal barrier of rotation. The internal barrier of rotation was especially useful for describing the conformation of the PCBs on a continuous scale.

In total 52 physico-chemical descriptors were compiled and analysed by PCA for the tetra- to hepta-chlorinated congeners. The structural variation within these compounds was condensed into four principal properties derived from a PCA for use as design variables in a statistical design to select congeners representative for these homologue-groups. The 20 selected PCBs have been applied to study structure-specific biochemical responses in a number of bioassays, and to study the biomagnification of the PCBs in various fish species.

QSARs were established using partial least squares projections to latent structures (PLS) for the PCBs potency to inhibit intercellular communication, activate respiratory burst, inhibit dopamine uptake in synaptic vesicles, compete with estradiol for binding to estrogen receptors, and induce cytochrome P4501A (CYP1A) related activities. By the systematic use of the designed set of PCBs the biological potency was screened over the chemical domain of the class of compounds. Further, sub-regions of highly potent PCBs were identified for each response measured. For risk assessment of the PCBs potency to induce dioxin-like activities the predicted induction potencies (PIPs) were calculated. In addition, two sets of PCBs were presented that specifically represent congeners of environmental relevance in combination with predicted potency to induce estrogenic and CYP1A related activities.

Keywords polychlorinated biphenyls, PCBs, physico-chemical properties, statistical design, multivariate, PCA, PLS, biomagnification, BMF, bioassay, CYP1A, SAR, QSAR, REPs, risk assessment

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Contents

1. List of Papers

This thesis is based on the following papers, which will be referred to in the text by their roman numerals.

I. Andersson P, Haglund P, Rappe C and Tysklind M. “Ultraviolet absorption characteristics and calculated semi-empirical parameters as chemical descriptors in multivariate modelling of polychlorinated biphenyls”. *J Chemometrics* 10:171-185, 1996.

II. Andersson P.L, Haglund P and Tysklind M. “The Internal Barriers of Rotation for the 209 Polychlorinated Biphenyls”. *Environ Sci & Pollut Res* 4:75-81, 1997.

III. Andersson P.L, Haglund P and Tysklind M. “Ultraviolet absorption characteristics of all 209 polychlorinated biphenyls evaluated by principal component analysis”. *Fresenius J Anal Chem* 357:1088-1092, 1997.

IV. Tysklind M, Andersson P, Haglund P, van Bavel B and Rappe C. “Selection of polychlorinated biphenyls for use in quantitative structure-activity modelling”. *SAR & QSAR in Environ Research* 4:11-19, 1995.

V. Andersson P.L, Berg A.H, Bjerselius R, Norrgren L, Olsson PE, Örn S and Tysklind M. “Uptake and elimination of selected PCBs in zebra fish, three-spined stickleback and arctic char after three different routes of exposure”. Submitted to *Arch Environ Contam Toxicol* 2000.

VI. Andersson P.L, van der Burght A.S.A.M, van den Berg M and Tysklind M. ”Multivariate modeling of PCB-induced CYP1A activity in hepatocytes from three different species: Ranking scales and species differences”. *Environ Toxicol Chem* 19:1454-1463, 2000.

2. Abbreviations

AM1	Austin Model 1
BMF	Biomagnification factor
CYP	Cytochrome P540
DDT	Dichlorodiphenyl trichloroethane
E2	17β-estradiol
EC_{50}	Effective concentration for 50% of maximal effect
ER	Estrogen receptor
EROD	Ethoxyresorufin-*O*-deethylase
Erot	Internal barrier of rotation
GC	Gas chromatography
GJIC	Gap junction intercellular communication
IC_{50}	Effective concentration for 50% of maximal inhibition
Kow	Octanol-water partition coefficient
MCF-7	Human breast cancer cells
MROD	Methoxyresorufin-*O*-demethylase
OH-PCB	Hydroxylated polychlorinated biphenyl
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxins
PCDF	Polychlorinated dibenzofurans
PCN	Polychlorinated naphthalene
PCQ	Polychlorinated quaterphenyl
PIP	Predicted induction potency
PLS	Partial least squares projections to latent structures
POP	Persistent organic pollutant
Q^2	Cross-validated explained variance
QSAR	Quantitative structure-activity relationship
REP	Relative effect potency
RMSEP	Root mean squared error of predictions
R^2Y	Explained variance of the dependent variable
SAR	Structure-activity relationship
TCDD	Tetrachlorinated dibenzo-*p*-dioxins
TEF	Toxic equivalency factor
TEQ	Toxic equivalent concentration
UNEP	United Nations Environmental Programme
UV	Ultraviolet absorption spectra

3. Introduction

In recent years, the use of quantitative structure-activity relationships (QSARs) to predict the fate, persistence, and biological effects of environmental contaminants has increased. QSAR approaches including multivariate data analysis in combination with statistical design have become extensively used. Using multivariate data analysis many potentially relevant property descriptors and biological activity measurements can be handled simultaneously. In environmental chemistry this approach has been successful since investigated contaminants often include many congeners with similar structural characteristics and modes of biological action. In the work described in this thesis, statistical design was used to select training and validation sets and multivariate techniques were used to model QSARs concerning various properties of the polychlorinated biphenyls (PCBs).

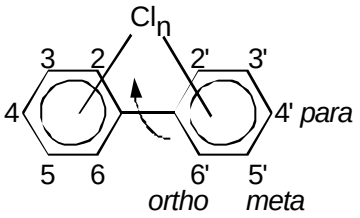

Figure 1. General structural formulae and substitution positions of the PCBs.

The PCBs comprise a group of 209 structurally different congeners with the empirical formula $C_{12}H_{10-n}Cl_n$ (n=1-10; see Figure 1). The environmental occurrence of PCBs was first reported in 1966 by Jensen, who found extremely high levels of PCBs in a white-tailed sea eagle found dead in the Stockholm archipelago. Today, PCBs can be found in all environmental compartments from the bottoms of the oceans to the aerial polar regions. The PCBs are spread into the environment from dumps, landfills, combustion processes, and from their use in various open and closed systems. They are lipophilic and enriched in adipose tissues of predators, mainly through consumption of contaminated food. The PCBs have also been shown to cause a multitude of toxic responses in wildlife and humans (Giesy and Kannan 1998; Safe 1994, van den Berg *et al*. 1998). The toxic effects of the PCBs were brought to public awareness by the Yusho incident in Japan 1968, where in a sudden epidemic in Western Japan, more than 1800 persons were injured due to consumption of contaminated rice oil (Kuratsune 1996). In Sweden and many other industrial countries, the production and use of PCBs have been strictly restricted since the 1970s.

The United Nations Environmental Programme (UNEP) has established a list of 12 classes of persistent organic pollutants, including the PCBs, along with substances such as the polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), dichlorodiphenyl trichloroethane (DDT), toxaphene, and dieldrin (UNEP report 1998). These substances are listed for global priority action to eliminate discharges, emissions, and losses.

3.1 Production and use

The commercial production of PCBs started in the late 1920s and dropped dramatically during the 1970s due to scientific and public concern. The total production of PCBs has been estimated at 1.5 million tonnes (de Voogt and Brinkman 1989). The Monsanto Industrial Chemicals Co. (St. Louis, Missouri, USA) was one of the largest producers and sold mixtures of PCBs under the name Aroclor until 1977. Trade names of other producers are Kanechlor (Kanegafuchi Chemicals Co., Japan), Clophen (Bayer A.G., Germany), and Fenclor (Caffaro, Italy). The production of PCBs involves batch chlorination of biphenyl and the congener pattern in the product is principally determined by the reaction time and the amount of chlorine. More than 140 congeners can be separated from the technical mixtures (Larsen *et al.* 1992). In addition, these mixtures also contain a number of contaminants in parts per million levels, such as PCDFs, polychlorinated quaterphenyls (PCQs) and poly-chlorinated naphthalenes (PCNs) (de Voogt and Brinkman 1989).

The commercial PCB products, such as the Aroclors, typically consist of 50 to 70 congeners. Most of these mixtures are liquids at room temperature. The physico-chemical properties of the commercial mixtures depend on the congener composition, but generally they are resistant to acids and bases, resistant to oxidation and hydrolysis, thermally stable, excellent electrical insulators, sparingly soluble in water and have low flammability (de Voogt and Brinkman 1989). These characteristics made them very useful in diverse industrial applications, such as liquid components of transformers, capacitors, heat-exchangers, and vacuum pumps. PCB mixtures have also been used in open systems, such as plasticizers, deinking solvents, water-proofing agents, sealing liquids, fire retardants and pesticides (de Voogt and Brinkman 1989).

3.2 Physico-chemical properties of individual PCBs

In 1980, Ballschmiter and Zell presented a numbering system for the 209 individual PCBs that follows the IUPAC rules (see Figure 1 and Table 1). Three years later, minor amendments to this system were suggested by Schulte and Malisch (1983). The molecular weights of the PCBs range from 188.7 to 498.7 based on the natural abundance of carbon, hydrogen, and chlorine (de Voogt and Brinkman 1989). The PCBs are soluble in organic solvents, oils and fats, but show an extremely low solubility in water, especially the more highly chlorinated biphenyls. In the literature, specific physico-chemical properties of individual PCBs may vary between measurements. These values are critical for modelling aspects such as the transport and fate, persistence, bioconcentration, and biological activity of the congeners.

An important physico-chemical characteristic of the PCBs is their ability to rotate around the phenyl-phenyl bond. The conformation of the PCBs has been shown to be correlated with their toxicity, strength of adsorption to surfaces, and partition between various media. Although the non-*ortho* PCBs are often described as “the coplanar congeners”, all PCBs regardless of substitution pattern, are twisted (McKinney and Singh 1981). The energy barrier of rotation increases as the number of chlorine atoms in *ortho* positions increases. The electron diffraction technique has been used to estimate the dihedral angles of some PCBs. For instance, Almenningen *et al.* (1985) reported this angle to be 44° for the non-*ortho* PCB 2 and Bastiansen (1950) reported 74° for the di-*ortho* PCB 4. The energy barrier of internal rotation for the tri- and tetra-*ortho* PCBs severely restricts their rotation (Kaiser 1974). Among the 209 PCBs, 19 are predicted to be atropisomers, i.e. they are conformationally stable and optically active under most environmental conditions (Kaiser 1974). The atropisomers can be isolated by liquid chromatography with chiral stationary phases (Haglund 1996). Further, the biological potency, both *in vitro* and *in vivo*, has been shown to differ between enantiomers of the same atropisomeric PCB (Püttmann *et al.* 1989).

Table 1. Systematic numbers of the PCBs (Ballschmiter and Zell 1980; Schulte and Malisch 1983).

no	structure	no	structure	no	structure	no	structure	no	structure
1	2	43	22'35	85	22'344'	127	33'455'	169	33'44'55'
2	3	44	22'35'	86	22'345	128	22'33'44'	170	22'33'44'5
3	4	45	22'36	87	22'345'	129	22'33'45	171	22'33'44'6
4	22'	46	22'36'	88	22'346	130	22'33'45'	172	22'33'455'
5	23	47	22'44'	89	22'346'	131	22'33'46	173	22'33'456
6	23'	48	22'45	90	22'34'5	132	22'33'46'	174	22'33'456'
7	24	49	22'45'	91	22'34'6	133	22'33'55'	175	22'33'45'6
8	24'	50	22'46	92	22'355'	134	22'33'56	176	22'33'466'
9	25	51	22'46'	93	22'356	135	22'33'56'	177	22'33'4'56
10	26	52	22'55'	94	22'356'	136	22'33'66'	178	22'33'55'6
11	33'	53	22'56'	95	22'35'6	137	22'344'5	179	22'33'566'
12	34	54	22'66'	96	22'366'	138	22'344'5'	180	22'344'55'
13	34'	55	233'4	97	22'3'45	139	22'344'6	181	22'344'56
14	35	56	233'4'	98	22'3'46	140	22'344'6'	182	22'344'56'
15	44'	57	233'5	99	22'44'5	141	22'3455'	183	22'344'5'6
16	22'3	58	233'5'	100	22'44'6	142	22'3456	184	22'344'66'
17	22'4	59	233'6	101	22'455'	143	22'3456'	185	22'3455'6
18	22'5	60	2344'	102	22'456'	144	22'345'6	186	22'34566'
19	22'6	61	2345	103	22'45'6	145	22'3466'	187	22'34'55'6
20	233'	62	2346	104	22'466'	146	22'34'55'	188	22'34'566'
21	234	63	234'5	105	233'44'	147	22'34'56	189	233'44'55'
22	234'	64	234'6	106	233'45	148	22'34'56'	190	233'44'56
23	235	65	2356	107	233'4'5	149	22'34'5'6	191	233'44'5'6
24	236	66	23'44'	108	233'45'	150	22'34'66'	192	233'455'6
25	23'4	67	23'45	109	233'46	151	22'355'6	193	233'4'55'6
26	23'5	68	23'45'	110	233'4'6	152	22'3566'	194	22'33'44'55'
27	23'6	69	23'46	111	233'55'	153	22'44'55'	195	22'33'44'56
28	244'	70	23'4'5	112	233'56	154	22'44'56'	196	22'33'44'56'
29	245	71	23'4'6	113	233'5'6	155	22'44'66'	197	22'33'44'66'
30	246	72	23'55'	114	2344'5	156	233'44'5	198	22'33'455'6
31	24'5	73	23'5'6	115	23'44'6	157	233'44'5'	199	22'33'4566'
32	24'6	74	244'5	116	23456	158	233'44'6	200	22'33'45'66'
33	2'34	75	244'6	117	234'56	159	233'455'	201	22'33'455'6'
34	2'35	76	2'345	118	23'44'5	160	233'456	202	22'33'55'66'
35	33'4	77	33'44'	119	23'44'6	161	233'45'6	203	22'344'55'6
36	33'5	78	33'45	120	23'455'	162	233'4'55'	204	22'344'566'
37	344'	79	33'45'	121	23'45'6	163	233'4'56	205	233'44'55'6
38	345	80	33'55'	122	2'33'45	164	233'4'5'6	206	22'33'44'55'6
39	34'5	81	344'5	123	2'344'5	165	233'55'6	207	22'33'44'566'
40	22'33'	82	22'33'4	124	2'3455'	166	2344'56	208	22'33'455'66'
41	22'34	83	22'33'5	125	2'3456'	167	23'44'55'	209	22'33'44'55'66'
42	22'34'	84	22'33'6	126	33'44'5	168	23'44'5'6		

3.3 Environmental occurrence

The PCBs are ubiquitous pollutants and the levels of PCBs generally increase from lower to higher trophic levels (Bright *et al.* 1995; Jansson *et al.* 1993; McFarland and Clarke 1989; Willman *et al.* 1997). The pattern of the PCBs found in biota does not resemble the composition of the commercial PCB products. PCBs released to the environment are partitioned between different media and transformed through a range of processes, such as photolysis, microbial activity, and metabolism. Among the 209 PCBs, McFarland and Clarke (1989) suggested 36 to be environmentally threatening due to their environmental prevalence, relative abundance in animal tissues, and potential toxicity. These 36 PCBs are listed in Table 2. Total PCB levels in muscle from herring caught along the Swedish coast ranged between 510 and 2400 ng/g lipid (Bignert *et al.* 1998). These values can be compared with the Swedish national limit for PCB 153 in fish products of 100 ng/g (Darnerud *et al.* 1995). For comparison, PCB 153 accounts for roughly 10 to 14% of total PCBs, and herring muscle consists of about 5 to 10% lipids (Atuma *et al.* 1996). Since the production and use of PCBs were restricted in most industrial countries, in the late 1970s, the levels in the environment have declined (de March *et al.* 1998; Sanders *et al.* 1994). However, the decrease in levels has been slower for the PCBs compared to the DDTs (Bignert *et al.* 1998). These authors concluded that most likely PCBs still enter the environment.

Table 2. Environmentally important PCBs (McFarland and Clarke 1989)

18	74	105	128	158	180
37	77	114	138	167	183
44	81	118	151	168	187
49	87	119	153	169	189
52	99	123	156	170	194
70	101	126	157	177	201

A retrospective study by Alcock *et al.* (1993) showed that the PCB levels in soil in the UK peaked during the late 1960s to early 1970s. The levels of PCBs have since then decreased to levels comparable with those found in the soil in the 1940s, i.e. 20-30 ng/g (dry weight). These authors also reported changes in the PCB patterns, towards greater proportions of highly chlorinated PCBs, in the most recent samples. In a sediment core from the northwestern Baltic Proper, the levels of PCBs peaked in the disk from 1978 (age range 1974-81) at 11 ng/g (dry weight) and decreased in the more

recent disk to 2.6 ng/g (Kjeller and Rappe 1995). A decreasing trend of PCB levels has also been observed in archived herbage samples (Jones *et al.* 1992), peat and sediment cores (Sanders *et al.* 1992, 1995), and stored air filter samples (Jones *et al.* 1995). Recent air samples collected around the Baltic Sea indicate a median current concentration of total PCBs of 57 pg/m^3 (Agrell *et al.* 1999). Slightly higher PCB levels (89-370 pg/m^3) in the air were found at sites near the Great Lakes (Hillery *et al.* 1997). The atmospheric levels of PCBs are correlated with temperature. Thus, higher concentrations of the highly chlorinated PCBs, especially, are found during the summer (Haugen *et al.* 1999; Hillery *et al.* 1997).

The levels in human tissues are also decreasing. A survey of human milk samples collected in Sweden between 1972 and 1992 showed a 65% decline in this period (Lundén and Norén 1998). The total concentration of PCBs in 1992 was 380 ng/g lipid and over the whole period PCBs 138, 153, and 180 were the most abundant in milk. Other PCBs abundant in human milk are PCBs 28, 52, 118, 156, and 167 (Lundén and Norén 1998). In a comparison of PCB and dioxin profiles in the general population of Sweden and Spain, the median level of total PCBs in adipose tissue was 1310 ng/g lipid (Wingfors *et al.* 2000). In this study, 31 congeners were quantified and the highest concentrations were found for PCBs 74, 99, 118, 138, 153, 156, 180, 183, 194, and 201. Further, the overall PCB levels in samples from two population groups, representing Sweden and Spain, did not differ, but showed differences in congener patterns, as evaluated by multivariate techniques (Wingfors *et al.* 2000).

3.4 Transport and fate

The global distribution of the PCBs suggests that air transportation of the compounds occurs. The fugacity of the PCBs, or their tendency to escape to another compartment, was fairly similar for soil, water, and sediment, but about a factor of ten lower in air (Mackay and Paterson 1991). According to model calculations by these authors (concerning hexachlorobiphenyls) most of the PCBs was found in soils and sediments. The pattern of PCBs in the environment varies due to source of PCBs and to physical, chemical, and biological transformation processes. Anaerobic dechlorination has been suggested to cause changes in PCB patterns in sediments from Hudson River, for instance (Brown *et al.* 1987). Furukawa *et al.* (1978) studied biodegradation of PCBs by two different bacterial species and concluded that degradation decreased as the number of chlorine atoms increased, that

PCBs with less than three *ortho* chlorine atoms are more slowly degraded, and that congeners lacking substituents on one ring are rapidly degraded. The PCBs have also been shown to degrade by photolysis (Hutzinger *et al*. 1972). The PCBs are photodecomposed by stepwise dechlorination towards the more photolytically stable, less heavily chlorinated PCBs (Ruzo *et al*. 1974a). In photolysis the least stable chlorine atoms are those in the *ortho* positions followed by those in the *meta* positions (Miao *et al*. 1999).

Many POPs, including the PCBs, are found at considerable concentrations in polar regions. The POPs are vaporised, condensed and consequently fractionated latitudinally due to differences in physico-chemical properties (Rappe 1974; Wania and Mackay 1993). According to the global fractionation theory, the pattern of PCBs should change in a south to north profile, the less heavily chlorinated congeners tending to become more abundant in the north. In moss samples from Norway, the highly chlorinated PCBs have declined more in the southern sampling sites than the northern sites, and Lead *et al.* (1996) concluded that this observation is consistent with the global fractionation theory. In the colder northern regions the lightly chlorinated PCBs are decreasing more rapidly as these congeners are revolatised faster from soil and vegetation and transported further north. However, the levels in biota from both southern and northern Sweden are declining at the same rate and, thus, Bignert *et al.* (1998) suggested a one-step long-range transport of the PCBs occurs rather than global fractionation.

3.5 Metabolism

Although the PCBs are considered extremely persistent compounds they undergo biotransformation. The biological half-lives of PCBs 138, 153, and 180 have been determined in a man exposed to labelled compounds (Bühler *et al.* 1988). PCB 153 was retained for the longest time in the blood, and the half-life of this compound was estimated to be 338 days. PCBs substituted in *meta* and *para* positions have been shown to be less susceptible to metabolic transformation than those lacking chlorines in these positions (Borlakoglu and Wilkins 1993). The PCB pattern in Clophen A50-treated mink was found to be dominated by congeners with more than four chlorine atoms and lacking unsubstituted vicinal *meta-para* positions (Bergman *et al*. 1992). The initial transformation processes of PCBs include oxidation by different cytochrome P450 isoenzymes (CYPs), e.g. CYP1A, CYP2B, and possibly CYP2C and CYP3A (Letcher *et al.* 2000). Different CYPs are

induced depending on the substitution pattern of the PCBs and the species studied (Ariyoshi *et al.* 1995, Ishida *et al.* 1991, Koga *et al.* 1995). PCBs are mainly metabolised via formation of arene oxides or by direct insertion of a hydroxy-group, as illustrated in Figure 2 (Koga *et al.* 1992). The intermediate arene oxide is subsequently rearranged to a hydroxylated PCB (OH-PCB) or further metabolised to a diol via secondary hydroxylation (Ariyoshi *et al.* 1997). Arene oxides may also react with glutathione, forming methyl sulfone PCBs, via a multistep mechanism involving the mercapturic acid pathway (Bakke *et al.* 1982). The arene oxides seem to be most commonly formed in the *meta-para* positions (Letcher *et al.* 2000). Due to 1,2-shifts (NIH-shifts), the hydroxy-group can be found in either *meta* or *para* positions and the adjacent chlorine atom may also shift position (Letcher *et al.* 2000). In rats exposed to PCBs 105, 118, 138, 153, 157, 183, and 187, NIH-shifts were observed for the five penta- and hexa-chlorinated congeners (Sjödin *et al.* 1998).

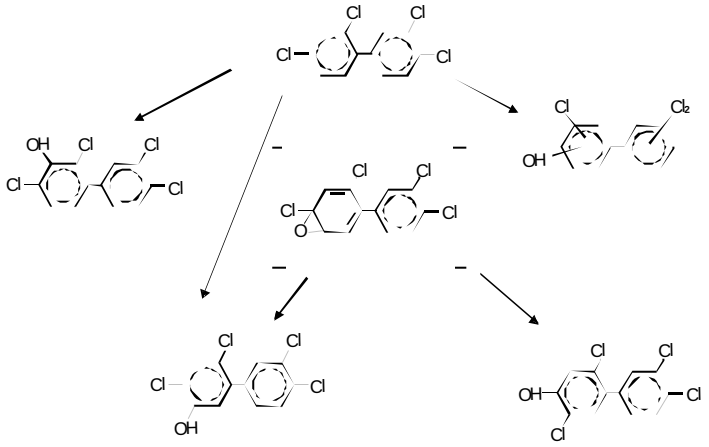

Figure 2. Metabolic pathways of PCB 66 in rat, suggested by Koga *et al.* 1992.

OH-PCBs have been found in various materials, such as excreta from rats and pigeons (Hutzinger *et al.* 1972), faeces of grey seals and guillemots (Jansson *et al.* 1975), blood of grey seals, plasma of humans (Bergman *et al.* 1994), and polar bear blood plasma (Sandau and Norstrom 1996). The retention of OH-PCBs in plasma seems to be selective and involve binding to thyroid hormone transporting proteins (Letcher *et al.* 2000). The retained OH-PCBs are most often substituted with the hydroxy-group in the *para* position and chlorine atoms in adjacent positions (Bergman *et al.* 1994). This pattern resembles thyroxine, a natural hormone and ligand to the thyroxine

transporting protein, transthyretin (Letcher *et al.* 2000). Methyl sulfone PCBs have also been detected in diverse materials, such as blubber of seals (Jensen and Jansson 1976), Clophen A50-treated mink (Bergman *et al.* 1992), and human blood plasma (Norén *et al.* 1999). These PCB metabolites have shown tissue-specific retention due to reversible protein binding (Letcher *et al.* 2000). The liver and lung appear to be organs with high specific retention of methyl sulfone PCBs, although these compounds have also been found in other tissues and fluids, including adipose and kidney tissues, blood, and human milk (Letcher *et al.* 2000).

3.6 Human health effects

Accidental exposure to PCBs through contaminated rice oil occurred in Japan, 1968, and in Taiwan, 1979. The resulting disease was called Yusho in Japan and Yu-cheng in Taiwan, i.e. “oil disease” in Japanese and Chinese, respectively. In addition to PCBs, other contaminants such as PCDFs and PCQs were identified in the rice oil (Miyata *et al.* 1985). The early symptoms of the victims included chloracne, increased eye discharge, swelling of the upper eyelids, fever, and vomiting (Kuratsune 1989). Infants from exposed mothers showed clinical symptoms similar to exposed adults and growth appeared to be disturbed in boys (Kuratsune 1996). Further, increased mortality from cancer of the liver and of the respiratory system was observed in males. The most severe effects observed in the victims were most probably related to the PCDFs in the rice oil (Kuratsune 1996). Occupational exposure to PCBs has been shown to cause toxic effects in workers who produced PCBs or utilised PCB-containing products. The effects included chloracne, diverse hepatic responses, eye irritation, and decreased birth weight in the offspring of exposed mothers (Safe 1994). Mortality, however, seems not to be increased even in highly exposed workers (Kimbrough *et al.* 1999). Increased incidences of specific cancer forms have been reported in occupationally exposed men and women, such as melanomas, liver, gall bladder, and bilary tract cancers, gastrointestinal tract cancer and hematologic neoplasms (Safe 1994). However, the data on cancer risk and occupational PCB exposure is inconclusive (Brouwer *et al.* 1998; Longnecker *et al.* 1997; Safe 1994).

The general human population is exposed to PCBs mainly through consumption of fish or other fat-rich food. The low environmental levels of PCBs are unlikely to cause adverse human health effects in adults (Safe 1994). However, possible links between increased incidences of breast

cancer and elevated levels of PCBs and pesticides have been discussed (Falck *et al*. 1992). Further, Jacobson and Jacobson (1997) found that prenatal exposure of PCBs were correlated with neurobehavioral effects in children from Michigan. These children were the offspring of fish-consuming Michigan mothers, who were compared with children from a general population in North Carolina. The former group was found to perform more poorly in a memory test (Jacobson and Jacobson 1997). Similar results have also been observed in The Netherlands, where reduced neonatal neurological optimality was correlated with increased pre- and early-neonatal exposure (Huisman *et al*. 1995). However, the nature of exposure is complex, and the cause of the observed developmental effects may be related to compounds other than the PCBs.

3.7 The TEF concept

The PCBs and PCDD/Fs exist as complex mixtures in the environment and induce several shared toxic responses. To estimate the relative potency of individual PCBs and PCDD/Fs and to assess the risks posed by these compounds, the toxic equivalency factor (TEF) concept has been adopted (e.g. Ahlborg *et al*. 1992, 1994; Safe 1990, 1994; van den Berg *et al*. 1998). The reference chemical used for this purpose is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is assigned the TEF-value 1. For environmental samples the toxic equivalent (TEQ) concentration can be calculated as the sum of the product of the concentration of each compound and its TEF-value. The rationale behind the TEF concept is the assumption that there is a common mechanism of action for these compounds via initial binding to the Aryl hydrocarbon (Ah) receptor. To judge the potential toxicity of the compounds, both *in vitro* and *in vivo* studies are used. The compounds assessed using the TEF concept must; 1) show structural similarities with PCDD/Fs, 2) bind to the Ah-receptor, 3) elicit Ah-receptor mediated biochemical and toxic responses, and 4) be persistent and accumulate in the food chain (van den Berg *et al*. 1998). The TEFs presented by van den Berg *et al*. (1998) were presented in three classes, describing toxic equivalent factors for mammals, fish and birds, see Table 3. In this evaluation of the TEFs, four non-*ortho* PCBs and eight mono-*ortho* PCBs were assigned TEFs. These PCBs are tetra- to hepta-chlorinated and possess chlorine atoms in both *para* positions and at least two *meta* positions.

Table 3. Toxic equivalency factors for PCBs (van den Berg *et al*. 1998)

PCB	Humans / mammals	Fish	Birds
77 (33'44')	0.0001	0.0001	0.05
81 (344'5)	0.0001	0.0005	0.1
105 (233'44')	0.0001	<0.000005	0.0001
114 (2344'5)	0.0005	<0.000005	0.0001
118 (23'44'5)	0.0001	<0.000005	0.00001
123 (2'344'5)	0.0001	<0.000005	0.00001
126 (33'44'5)	0.1	0.005	0.1
156 (233'44'5)	0.0005	<0.000005	0.0001
157 (233'44'5')	0.0005	<0.000005	0.0001
167 (23'44'55')	0.00001	<0.000005	0.00001
169 (33'44'55')	0.01	0.00005	0.001
189 (233'44'55')	0.0001	<0.000005	0.00001

3.8 Quantitative structure-activity relationships

The 12 persistent organic pollutants listed by the United Nations in the Global Convention on Persistent Organic Pollutants actually comprise thousands of compounds (UNEP report 1998). The toxaphenes alone include several thousand compounds, commercial mixtures of chlordane include more than 140 compounds, and PCBs and PCDD/Fs can theoretically consist of 209 and 210 congeners, respectively. Fortunately, a large number of these compounds have never been produced or been formed by natural processes. Further, many chemicals are not persistent in the environment, because of their susceptibility to chemical, physical or biological degradation. Nevertheless, organisms in most ecosystems are exposed to complex mixtures of compounds, which individually or in total constitute a risk to health. A multitude of toxicological effects in humans and wildlife has been linked to the increased load of pollutants. The mechanisms underlying these effects are complex and thus many physico-chemical features of the target compounds must be quantified to estimate their potency.

A challenge often faced in environmental chemistry is to correlate the chemical structure of the compounds being investigated with their toxicological activity, i.e. to establish QSARs. A QSAR is a mathematical expression where variables describing chemical properties are related to results from biological tests. Thus, quantified variations in chemical structure are related to variations in the biological activity. Pioneering work

in QSAR methodology was done in the 1960s by Hansch and co-workers in the areas of drug design and pesticide research (Hansch *et al.* 1962). However, as early as 1868 Crum-Brown and Fraser published a paper on structure-activity relationships (SARs) in which water solubility was correlated to toxicity of alkaloids. The SAR approach can also be used to study other properties, such as retention times in chromatographic systems, thermal stability, or photolytic degradation. These phenomena involving structure-property relationships will however not be discussed in detail in the present thesis.

3.9 Aims and scope

The PCBs have been shown to evoke various toxic effects in exposed organisms. A systematic approach is warranted for the risk assessment of the PCBs, as it is neither economically nor practically feasible to study all the congeners individually. In order to assess the environmental impact of the PCBs, a QSAR methodology described by Eriksson (1991) has been adopted. In brief, this strategy includes the following six steps: classification of the chemicals, structural description, selection of training sets, biological testing, model development, and validation and prediction, see Figure 3. The work described in this thesis has been focused on the 154 tetra- to hepta-chlorinated congeners to increase the resolution of the developed QSAR models. These groups of homologues were considered to be the most relevant, due to their environmental abundance, persistence and toxicity. A set of measured and calculated physico-chemical descriptors was compiled in Paper I. The ultraviolet absorption spectra and the internal barriers of rotation were then studied in more detail (Papers II and III). Further, based on the compiled physico-chemical descriptors and statistical design, a set of 20 PCBs was selected for training and validation of SAR and QSAR models (Paper IV). In Papers V and VI, SARs and QSARs were established linking structural features with biomagnification of the PCBs in various fish species, and induction of CYP1A activity in hepatocytes from chicken, monkey, and pig. In addition, new sets of PCBs are presented in this thesis for optimisation and validation of QSARs, especially for studies concerning specific parts of the chemical domain of the PCBs, see Figure 3.

The main aim of the work presented in this thesis was to achieve a better understanding of the structural characteristics and variation of the PCBs, and to develop QSARs with high predictive capacity. The structural variation of the PCBs is hypothesised to be correlated with the chemical and

biological properties of the compounds. Many features of the compounds are thought to influence the studied activities, and thus multivariate data analysis has been used for physico-chemical characterisation as well as QSAR modelling.

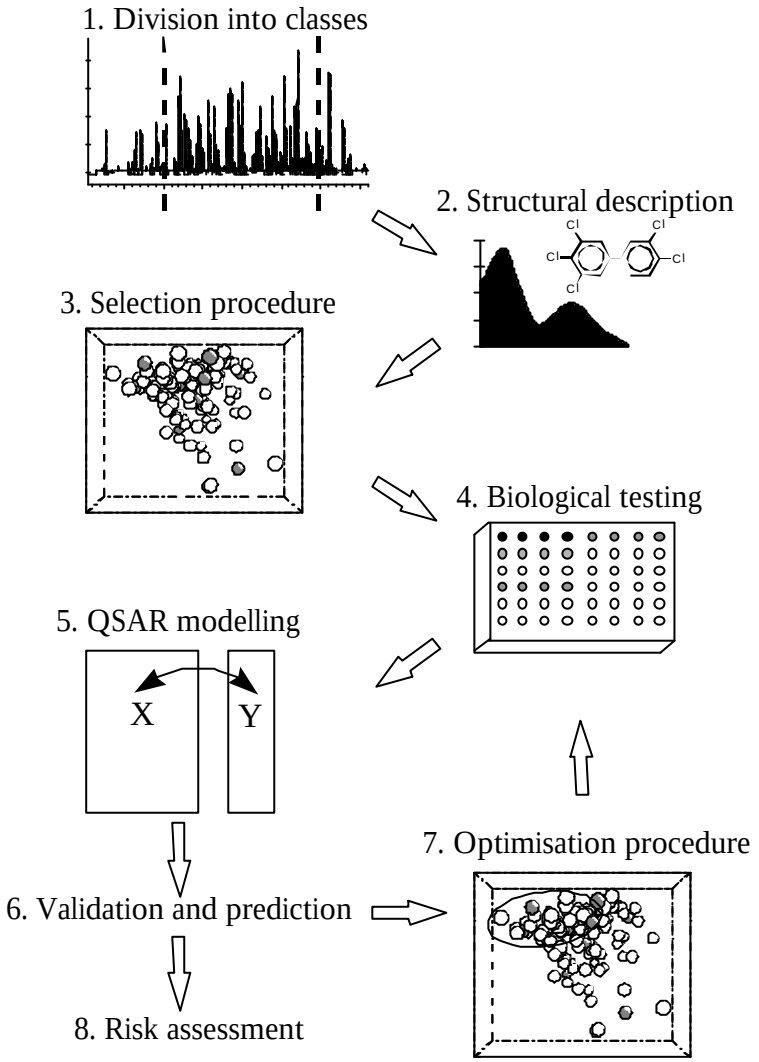

Figure 3. The QSAR strategy applied in this thesis: 1) Classification of the chemicals, 2) Physico-chemical characterisation, 3) Selection of representative training compounds, 4) Biological activity testing, 5) Establishment of the QSAR model, 6) Validation of the QSAR model and predictions of untested compounds, 7) Optimisation of the QSAR model, 8) Risk assessment of the studied class of compounds.

4. Multivariate methods and statistical design

Chemometric approaches including multivariate methods and statistical design have proved to be useful in environmental QSAR studies (Eriksson and Hermens 1995). Principal component analysis (PCA) and partial least squares projections to latent structures (PLS) are multivariate projection methods that have been used for pattern recognition and cluster analysis, physico-chemical characterisation of chemical substances and complex mixtures, structure-activity modelling, and to predict properties of unknown compounds or samples. Using sophisticated modern techniques, such as semi-empirical calculations, near infrared spectroscopy, and multidimensional chromatography, large data matrices are compiled that reflect physico-chemical characteristics of the compounds. These data can often be generated relatively cheaply and quickly for large numbers of compounds, in contrast to biological data, which are often time consuming and expensive to generate. Thus, the number of substances included in the biological testing protocol has to be heavily reduced. The representativeness of the compounds selected for testing is crucial and can be optimised by using PCA in combination with statistical experimental design (Jonsson *et al*. 1989). In this thesis, PCA has been used in Papers I, III, IV, VI and PLS in Papers I and VI.

4.1 Principal component analysis

PCA provides a means by which a multivariate data matrix may be analysed and interpreted by visualising dominating patterns and major trends in large data sets (Jackson 1991). The variation in any number of descriptors is projected into a few descriptive and uncorrelated (orthogonal) principal components. Further, PCA can handle situations where there are more variables than objects. The general patterns and the correlations among the objects, as well as the descriptors, are easy to interpret in graphical representations. In this project, the analysed data matrix usually included the 154 tetra- to hepta-chlorinated biphenyls as the objects and 52 physico-chemical variables as the descriptors.

In PCA the original data matrix, X, is re-expressed as a unity column vector times a mean row vector containing the average values of all descriptors, plus the product of T and P', and a residual matrix E ($X=1\overline{x}+TP'+E$). The T and P' matrices include the object scores and the descriptor loadings, respectively. Prior to the analysis, the data can be pre-processed by means of

auto scaling and mean centering. By auto scaling, the variables are scaled inversely to their standard deviation to give each variable equal importance in the model. The first principal component (PC), i.e. column in the T matrix, provides the best linear summary of X. The second PC, orthogonal to the first, accounts for the next largest variation, and so forth. The number of significant PCs should always be smaller than the number of objects and descriptors and is determined by using cross-validation (Wold 1978). Cross-validation provides a technique for obtaining a model with optimal predicting power without overfitting. The scores and the loadings can be plotted to overview the relation between the objects and the variables, respectively. By comparing the score plot and the loading plot, the relation between the chemicals and their structural descriptors can be derived. Thus, from a multi-dimensional space the major patterns in the data can be visualised in a few plots.

4.2 Partial least squares projections to latent structures

Two matrices, X and Y, are considered simultaneously in PLS (Dunn *et al.* 1984). Latent variables for the X and Y matrices, together with a relationship between them are calculated. In this thesis, PLS has been used to establish QSARs with 52 physico-chemical descriptors for the PCBs as the X-matrix and various biological response variables as the Y-matrix. The PLS method can handle several response descriptors and models with more descriptors than objects. Analogously with PCA, the data can be pre-processed by autoscaling and mean centering. The PLS method is presented in Figure 4 by a geometrical representation. The physico-chemical variation in the X-matrix is projected onto a subspace, T. Simultaneously, the variation in the biological response is projected onto the same subspace. The scores are calculated to approximate the variation in X but also to be well correlated with Y. The model is calculated to enable predictions of the biological response to be made from the physico-chemical data. For an untested compound the t-scores are calculated from the X-matrix and through the Y-score vector, u, the biological activity can be predicted, see arrows in Figure 4. A default probability level for compounds included in the models (members) was set to 0.05 (SIMCA).

As described for PCA, the significance of each model dimension is validated by using cross-validation (Wold 1978). The predictive power of the PLS model can be estimated from the prediction error sum of squares (PRESS). PRESS is transformed to Q^2, a dimension-less term calculated as $Q^2 = 1 -$

PRESS/SSY (Wold and Eriksson 1995). SSY equals the initial sums of squares for the dependent variable. Q^2 is referred to as the cross-validated explained variance and is a companion parameter to the explained variance, R^2. A more demanding validation of the predictive power of the model is to use an external validation set. The error in the predictions of these compounds can be estimated by calculating the root mean squared error of prediction (RMSEP). RMSEP is calculated using data from the validation set according to RMSEP = $(PRESS/N)^{1/2}$, where N equals the number of compounds in the validation set.

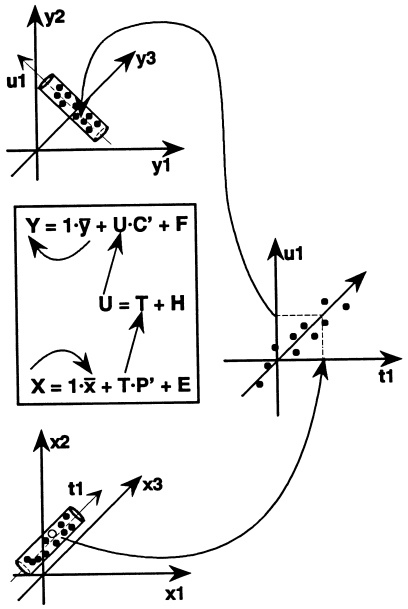

Figure 4. A geometrical representation of the PLS method (Eriksson 1991). The arrows indicate how the chemical data can be used to predict the dependent response for an untested object.

4.3 Statistical design

The selection procedure is a crucial step in the development of a QSAR (Tosato *et al.* 1990). A QSAR has only local validity and thus the studied compounds must be structurally similar. The training set must span the chemical domain of the studied class of chemicals to make interpolations possible. Further, the training set should be complemented by a validation set, which also gives a balanced representation of the chemical variation in the studied class (Eriksson and Hermens 1995). By using statistical

experimental design, such as factorial design or fractional factorial design, schemes are generated that introduce systematic variation of several factors or variables simultaneously (Box *et al.* 1978). In a factorial design the variables are assigned a certain number of levels, usually two or three, and the experiments are performed in all possible combinations. In the design illustrated in Figure 5, each factor is set at two levels (+ or -) and three factors are studied at the same time. Eight independent experiments are given by this 2^3 factorial design. A fractional factorial design is useful when the number of experiments must be low but many variables may be significant (Box *et al.* 1978). In the fractional factorial design, the original factorial design is divided into two balanced sets. Further, to capture curvature in the data and to cover the interior part of the experimental space, several centre points can be added with median values in all variables. These can also be used to estimate the statistical variation in the experiments.

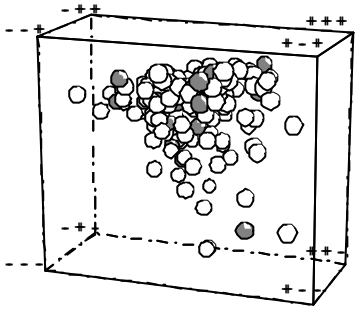

Figure 5. A 2^3 factorial design covering the chemical domain of the PCBs as described by the first three principal components. From each corner of the cube one compound is selected to represent that specific region.

In QSARs, the suggested experiments are the studied compounds and the factors are the physico-chemical characteristics. By using PCA the score values or the principal properties of the compounds can be calculated and applied as factors in the design (Skagerberg 1989). Compounds can then be selected with principal properties as close as possible to the design points, see Figure 5. By this method the entire chemical variation is captured in a balanced set of compounds. In addition, D-optimal designs have been shown to be useful when the physico-chemical domain of the compounds has constraints. D-optimal designs can span a constrained variable space and their goodness can be compared in terms of G-efficiency (Baroni *et al.* 1993; MODDE).

5. Physico-chemical descriptors and characteristics

The chemicals studied in a QSAR should have similar chemical and structural features as well as mode of biological action. It is assumed when developing QSARs that the factors underlying the studied biological mechanism are captured in the compiled set of physico-chemical descriptors. It is thus crucial to find descriptors that describe the chemical and structural variation of the compounds, i.e. their steric, hydrophobic, and electronic properties. In order to describe these features, a multitude of descriptors is thought to be needed (Hellberg 1986; Sjöström and Eriksson 1995). However, according to a recent review of SARs in environmental sciences, a good model can describe the biological activity with not more than three descriptors (Nendza 1998). Further, Nendza (1998) states that the predictive power of a SAR model decreases as the number of descriptors increases. Clearly, fundamentally different approaches have been suggested. However, this thesis is based on the hypothesis that multivariate chemical data as well as biological data are needed to establish QSARs with high predictive power.

The descriptors can be divided into physico-chemical, structural, topological, electronic, and geometric parameters (Jurs *et al*. 1995). Examples of physico-chemical parameters are various partition coefficients, density, melting point, boiling point, and reduction potential. Structural descriptors, such as the Hammet constant and fragment constant, summarise the frequency of functional groups substituted on core structures. The Hammet constant represents the contribution of substituents to the charge distribution whereas fragment constants describe the significance of certain fragments of the molecule. Topological descriptors are representations of the whole molecule and encode size, shape, or branching. Examples of such descriptors are molecular connectivity indices and Kappa indices. Electronic features of the compounds can be calculated by various methods from experimental to quantum chemical techniques. A number of electronic descriptors reflect intermolecular forces, such as dipole moments, dispersion, and hydrogen bonding. The energies of the highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO) are often used in QSARs. Another set of descriptors includes those related to the three-dimensional shape of the molecules. Examples of such descriptors are total surface area, total molecular volume, and van der Waals volume.

Table 4. The 52 physico-chemical parameters applied in the QSARs.

	Descriptor	Abbreviation	Reference
1	Binding energy	EB	Paper I
2	Isolated atomic energy	EIA	Paper I
3	Electronic energy	EE	Paper I
4	Core-core interaction energy	ECCI	Paper I
5	Heat of formation	Hf	Paper I
6	Internal barrier of rotation	Erot	Paper II
7-10	Dipole moment point-charge	PC x,y,z,tot	Paper I
11-14	Dipole moment hybridisation	Hyb x,y,z,tot	Paper I
15	Ionisation potential	Ip	Paper I
16	Electron affinity	Ea	Paper I
17	Absolute hardness	η	Paper I
18	Absolute electronegativity	χ	Paper I
19	Molecular Polarisability	Mol P	Ong, 1991
20	GC retention time	RRT1	Erickson, 1997
21	GC retention time	RRT2	Erickson, 1997
22	GC retention time	RRT3	Fischer, 1988
23	GC retention time	RRT4	Mullin, 1984
24	GC response factor	RRF4	Mullin, 1984
25	GC response factor	RRF1	Cooper, 1985
26	Octanol/Water partition	lgKow1	Hawker, 1988
27	Octanol/Water partition	lgKow2	Brodsky, 1988
28	Water solubility	lgSw	Brodsky, 1988
29	Henrys Law Constant	HLC1	Sabljic, 1989
30	Henrys Law Constant	HLC2	Dunnivant, 1992
31	Total Surface Area	TSA	Hawker, 1988
32-52	UV-spectra	200-300	Paper III

In this thesis, the chemical and structural descriptors are ascribed physico-chemical features, and classified, according to their origin, as non-empirical, semi-empirical, or empirical. Complete empirical data in the literature for all 209 PCBs are limited. Although PCA and PLS can handle missing data, these should ideally be uniformly spread in the data set, which is not always the case. The review on physico-chemical properties of PCBs by Mackay *et al*. (1992) included values for only 71 of the 209 PCBs. Thus, the use of non- and semi-empirical data offers an attractive complement to the empirical data. Non-empirical descriptors were used in Paper IV and these included the number of chlorine atoms in the *ortho* positions and ten indicator variables. The indicator descriptors were constructed to reflect the substitution pattern of the congeners so that chlorine atoms were assigned the value 1 and hydrogens 0 for each of the ten positions open for substitution. The data matrix applied for QSAR modelling in Papers I and VI

includes the 52 descriptors shown in Table 4 and is discussed in detail below.

5.1 Semi-empirical descriptors

The quantum chemical equations are complex and only molecular structures for very small systems can be calculated. Approximations of the Schrödinger wave function are required to describe molecules containing more than one atom by quantum chemical methods. The *ab initio* and semi-empirical approaches provide two different means of calculating molecular orbitals based on approximations of the Schrödinger wave function. In the *ab initio* methods, based solely on quantum mechanics, the orbitals in the system are approximated using different basis sets. The semi-empirical methods are parameterised by experimental data or data from *ab initio* calculations. Examples of semi-empirical methods are the intermediate neglect of diatomic overlap (INDO), the modified neglect of diatomic overlap (MNDO), the Austin Model 1 (AM1) (Dewar *et al.* 1985), and the parametric method 3 (PM3). These theoretical models all consider the ground state of the molecule in gaseous phase. The semi-empirical methods may yield different results, and derived descriptors should therefore be used independently and not be compared. In general, the *ab initio* methods are more precise than the semi-empirical methods, but also require more computation. For QSAR modelling with large classes of chemicals, the semi-empirical approach is advantageous as the calculations are relatively fast.

The three semi-empirical methods MNDO, AM1, and PM3, have been evaluated by comparing their capacity to find an accurate conformation of the biphenyl (Mulholland *et al.* 1993). The AM1 method showed results close to the experimental values and agreed well with the *ab initio* method STO-3G (Gaussian-80) (Mulholland *et al.* 1993). More recent studies on rotational barriers for conformationally constrained PCBs, however, have shown that AM1 fails to reproduce results from experimental measurements and *ab initio* calculations (Biedermann and Agranat 1999; Krupcik *et al.* 1995; Nezel *et al.* 1997). Although the AM1 method may yield quantitatively false results, the qualitative and relative ranking of rotational barriers for PCBs appears to be accurate (Andersson *et al.* 1999a; Nezel *et al.* 1997). Based on the results from Mulholland *et al.* (1993) and the extensive use of the AM1 method in previous studies, it was selected here for the semi-empirical calculations. The AM1 method has been used to study the relative stability of PCBs (Mulholland *et al.* 1993) and PCDDs (Huang *et al.* 1996),

for conformational analysis of diverse halogenated biphenyls (Nezel *et al*. 1997; Tang *et al*. 1991; Zimmermann *et al*. 1994), and to derive descriptors for structure-property (Makino 1998a,b; Ong and Hites 1991), and structure-activity modelling of various POPs (Lynam *et al*. 1998; Nevalainen and Kolehmainen 1994). In the X-matrix shown in Table 4, descriptors 1-18 originate from AM1 calculations. All calculations were performed using the HyperChem program package (HyperChem 2).

The calculated semi-empirical descriptors include diverse molecular energies, dipole moments, and measures of reactivity. The total energy of the molecule can be divided into the binding energy and the isolated atomic energy. The former reflects the number of chlorine atoms and is defined as the total energy of the system minus the isolated atomic energy. A summation of the core-core interaction energy and electronic energy terms gives the total energy of the system. The interaction between atomic cores is a positive parameter unlike the negative electronic energy. The heat of formation was calculated by subtracting atomic heat of formation values from the total energy. The internal barrier of rotation (Erot) was also calculated based on the total energy of the system. Dipole moments were calculated as a measure of the charge distributions of the molecules. These were calculated by two different approximations of the charge distribution, viz. point-charge and sp-hybridi-sation, in the x-, y-, z-directions and as a total.

The measures of reactivity include the energy of the frontier orbitals, HOMO and LUMO, and combinations of them. HOMO and LUMO can be approximated to the negative ionisation potential and the negative electron affinity, respectively (Pearson 1986). Further, absolute hardness and absolute electronegativity are defined as half of the difference, and of the sum, of the ionisation potential and the electron affinity, respectively (Pearson 1986). The absolute hardness of the PCBs varies depending on the number of chlorine atoms and the number of *ortho* substituents, see Figure 6. The absolute electronegativity is a measure of the electron attraction tendency and can be correlated with the negative chemical potential (Schüürmann 1990). The chemical hardness or softness of a molecule can be correlated with the absolute hardness (Pearson 1986). Molecules with a small HOMO-LUMO gap have small excitation energies to the manifold of excited states, therefore soft molecules, with a small gap, will be more polarisable than hard molecules (Pearson 1986). HOMO and LUMO have been frequently used as physico-chemical descriptors of electronic features

in QSAR studies for POPs (Kobayashi *et al.* 1992; Lynam *et al.* 1998; Nevalainen and Kolehmainen 1994; Tysklind *et al.* 1992; Veith *et al.* 1995). In addition, the molecular polarisability reported by Ong and Hites (1991) was calculated using the AM1 method.

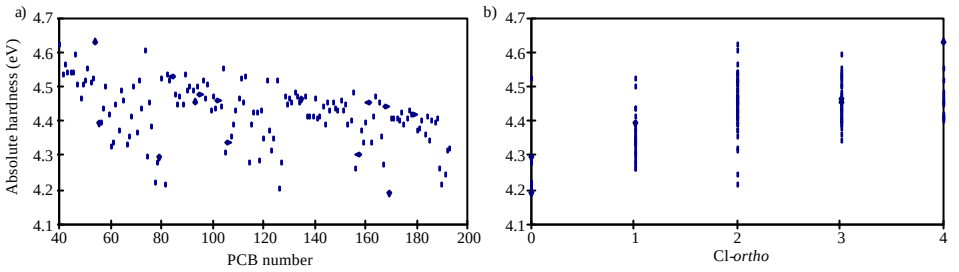

Figure 6. The absolute hardness of the PCBs plotted versus a) PCB number and b) number of *ortho* chlorine atoms (Cl-*ortho*).

5.1.1 Internal barrier of rotation

A few empirical estimations of the Erot values of PCBs have been reported, calculated from data on the enantiomerisation of chiral PCBs in combination with enantioselective gas (GC) or liquid chromatography (LC) (Harju and Haglund 1999; Schurig *et al.* 1995; Schurig and Reich 1998). The Erot values have also been calculated by *ab initio* methods (Biedermann *et al.* 1997; Biedermann and Agranat 1999; McKinney *et al.* 1983; Nezel *et al.* 1997). The results from the *ab initio* calculations generally agree well with experimental measurements. The *ab initio* calculations are time consuming, so the Erot has only been reported for a few PCBs. The Erot values for PCBs have also been determined using the semi-empirical methods INDO (Cullen and Kaiser 1984), MNDO (Sassa *et al.* 1986), and AM1 (Nezel *et al.* 1997; Tang *et al.* 1991; Zimmermann *et al.* 1994). A comprehensive report, including 150 PCBs, was published by Cullen and Kaiser (1984).

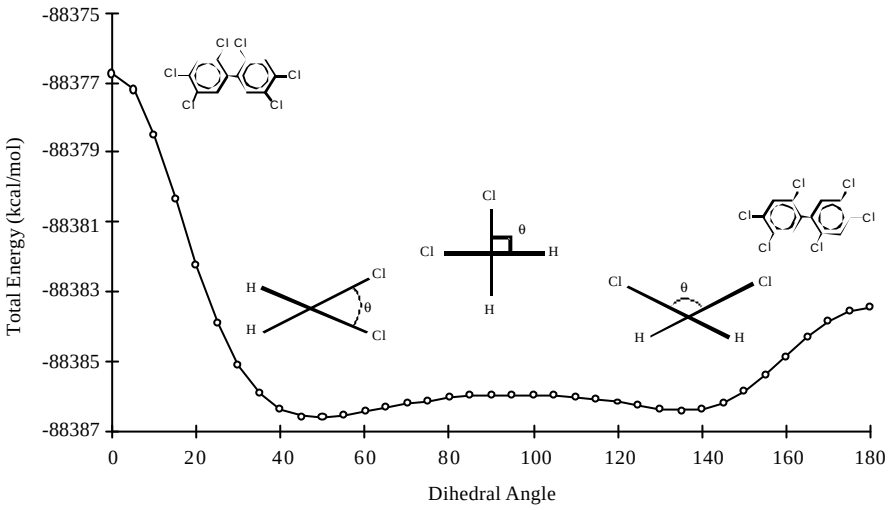

Figure 7. The total energy of PCB 153 versus dihedral angle calculated every 5th degree. Also illustrated are the structural formulae of the two forced planar states, i.e. syn- (0°) and anti-forms (180°), and the dihedral angles (θ) of the twisted conformation in syn- and anti-form and at 90°. The highest total energy of the forced planar states is found in the syn-form.

The internal barrier of rotation of all 209 PCBs, as calculated by AM1, is presented in Paper II. The Erot was defined as the difference in total energy between a constrained planar state and an optimised twisted conformation, see Figure 7. The total energy of the forced planar conformation was calculated for both the syn- (0°) and anti-forms (180°). The total energy of the anti-form was found to be lower and thus used in the subsequent calculations. In contrast, the prevailing conformation of twisted PCBs is the syn-form (Bastiansen 1950; Dynes *et al*. 1985; Römming *et al*. 1974). This is assumed to be due to non-bonding attractive forces between the chlorine atoms in *ortho* positions (Dynes *et al*. 1985). The AM1 calculations agreed well with these findings, as can be seen in Figure 7. The Erot values of the PCBs were found to vary between 8 and 480 kJ/mol from the non- to the tetra-*ortho* substituted biphenyls. For PCBs with vicinal *ortho*-*meta* chlorine atoms, the *meta* chlorine prevents the outward bending of the *ortho* substituent in the planar transition state. This so-called buttressing effect increased the Erot value by 4 to 31 kJ/mol per added chlorine atom in a buttressing *meta* position, see Figure 8.

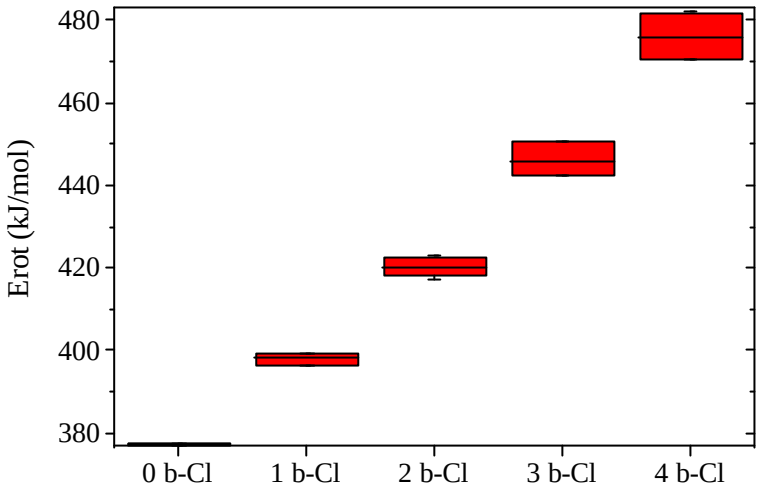

Figure 8. The significance of buttressing chlorine atoms (b-Cl) for the internal barrier of rotation of tetra-*ortho* PCBs. The Box and Whisker plots show the median, lower and upper quartiles, and the 95% confidence limits.

5.2 Empirical descriptors

In total 13 descriptors were taken from the literature, see Table 4. The total surface area and the molecular polarisability are derived from calculations, but the remainder are based on empirical measurements. However, the only true empirical descriptors, covering all 209 PCBs, are the gas chromatographic relative retention times and molar relative response factors (electron capture detector) determined by Mullin *et al*. (1984). Almost complete are the relative retention times reported by Fischer and Ballschmiter (1988) and the relative response factors determined for an electron capture detector by Cooper *et al*. (1985). In addition, artificial relative retention times were included in the X-matrix (Erickson 1997). These were derived from measured GC-retention times for all symmetric PCBs, followed by combining the half-ring retention times (half of the retention time of the symmetric PCB) to yield the retention times for all 209 PCBs. Altogether, the four relative retention times in the X-matrix reflect the interaction between PCB and GC coatings of widely different polarity, viz. dimethyl-polysiloxane (Erickson 1997), methyl-5% phenyl-polysiloxane (Mullin *et al*. 1984), 50% n-octylmethyl-polysiloxane (Fischer and Ballschmiter 1988), and 50%-cyanopropylphenylmethyl-polysiloxane (Erickson 1997).

Octanol-water partition coefficients (Kow) and water solubility were also included in the X-matrix. The Kow values reported by Hawker and Connell (1988) were calculated for all 209 PCBs based on a linear regression model including measured Kow and calculated total surface area data. The total surface area was calculated by assuming the molecules to be planar. Brodsky and Ballschmitter (1988) predicted Kow and water solubility for 154 PCBs from a model based on retention data for 87 PCBs. Further, air-water partition coefficients, reflected by the Henry's law constant (HLC) were predicted by Sabljic and Guesten (1989) for 146 PCBs using molecular connectivity indices. The second HLC in the X-matrix included all 209 PCBs and was derived from a QSPR model (Dunnivant *et al*. 1992).

5.2.1 Ultraviolet absorption spectra

Empirical physico-chemical descriptors for all PCBs were derived by measuring the ultraviolet absorption (UV) spectra between 200 and 300 nm in iso-octane (Paper III). The UV-spectra were digitised and collected every fifth nm to yield 21 descriptors. The main features of the UV-spectra of PCBs have previously been described in the literature (Curtis *et al*. 1967; Fenton 1969; MacNeil *et al*. 1976; Sundström 1973). The PCBs display two major absorption maxima, viz. the main-band (200-220 nm) and the κ-band (240-270 nm), see Figure 9. The main band is attributed to resonance in the bensenoid skeleton and is hence found for all congeners. The κ-band is most distinct in the spectra of the non-*ortho* PCBs. This second band is attributed to conjugation between the phenyl rings and was identified for more than 50 of the 209 PCBs. The molar extinction coefficients of this band were highest in the non-*ortho* PCBs, followed by the mono- and di-*ortho* PCBs. In addition, a third band was observed at about 220 to 240 nm, in-between the main band and the κ-band, see PCB 114 in Figure 9. This third band was found mainly for mono-*ortho* PCBs. The spectral information was interpreted by using PCA for all PCBs as well as sub-groups of the compounds (Paper III). In the model including all PCBs, the molecules ability to adopt a planar conformation was captured. Congeners with a clear κ-band separate in the first principal component from the majority of the PCBs. Most PCBs show a less characteristic spectrum and disperse only to a limited extent in the second component, depending on their molecular size. Two additional PCA models were calculated, to cover other properties of the PCBs that influence the UV-spectra, besides the number of *ortho* chlorine atoms. These models included the non- and mono-*ortho* substituted congeners. The spectra of

these congeners were significantly influenced by a *para-para* substitution pattern, as shown by the PCA (Paper III). Chlorine atoms in these positions have electron donating properties, which may induce a double-bond character in the inter-ring carbon-carbon bond (Ruzo *et al*. 1974b), shifting the conjugation band towards longer wave lengths (a bathochromic shift). In addition, the main band showed a bathochromic shift for the highly chlorinated non- and mono-*ortho* substituted PCBs.

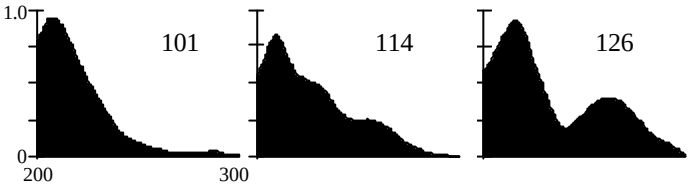

Figure 9. The UV-spectra in the range 200-300 nm for PCBs 101, 114, and 126.

5.3 Physico-chemical descriptors - an update

The commercial availability of all 209 PCBs has facilitated studies of their physico-chemical characteristics. Bush and Barnard (1995) measured gas phase infrared spectra of all 209 PCBs using GC linked with Fourier transform infrared detection. These spectra were reported to be very characteristic and may thus provide useful descriptors for QSAR and QSPR studies. Bolgar *et al*. (1995) measured the melting points, relative retention times (on two different GC columns), electron capture relative response factors, infrared spectra, and electron impact mass spectra for all PCBs. Further, Haglund and Harju (1998) reported electron impact mass spectrometric relative response factors and fragmentation data for all PCBs. Another complete set of data are the relative retention times measured on three types of stationary phases, viz. 5% diphenyl polydimethylsiloxane (non-polar), 10% permethylated cyclodextrin polydimethylsiloxane (chiral), and 50% liquid-crystalline polydimethylsiloxane (liquid-crystal) (Harju *et al*. 1998). Further, Frame (1997) presented a relative retention time database that included all 209 PCBs and 20 different stationary phases.

The use of semi-empirical molecular orbital methods has increased, providing a tool to derive complete physico-chemical data for large groups of compounds. Saito and Fuwa (2000) calculated the heat of formation, standard entropy, and specific heat capacity for PCBs and PCDD/Fs using descriptors derived from PM3 calculations. Parameters from AM1

calculations, such as the molecular weight, heat of formation, solvent accessible surface area, ionisation potential, electron affinity, and dipole moment were used to predict the aqueous solubility for 136 PCBs (Makino 1998a) and the octanol-water partition coefficients for 139 PCBs (Makino 1998b). Further, the calculated Gibbs free energies of formation are also available for all PCBs (Holmes *et al*. 1993).

5.4 Physico-chemical characterisation of PCBs

The 52 physico-chemical descriptors presented in Table 4 were used to summarise the structural and chemical variation of the PCBs (Paper I). The PCA model including the 154 tetra- to hepta-chlorinated biphenyls explained 73% of the variation with four principal components. The four PCs individually explained 35%, 26%, 8%, and 5% of the variation. The score- and the loading plots of these PCs are shown in Figure 10. The first PC generally reflects the size or number of chlorine atoms in the compounds. The tetra-chlorinated biphenyls, such as 46, 53, and 54, had low score values in PC1 compared to the hepta-chlorinated biphenyls, such as 189, 191, and 192, which all showed high score values in PC1. The second PC was clearly related to the number of chlorine atoms in the *ortho* positions. The coplanar PCBs, e.g. 77, 81, 126, and 169 had low score values, and the tetra-*ortho* PCBs, e.g. 54, 104, and 155, all had high score values. Descriptors with high influence in the first PC included the heat of formation, isolated atomic energy, electronic energy, Kow, and relative retention times. In the second PC, significant descriptors included the Erot, ionisation potential, and the UV-spectra between 250 and 300 nm. Thus, descriptors related to the molecular size showed the largest contribution in the first PC and those related to the conformation of the congeners were most significant in the second PC. However, most descriptors were not solely related to one characteristic of the compounds, such as the size or conformation, but to combinations of many features. Descriptors such as the isolated atomic energy, heat of formation, absolute hardness (Figure 6), and core-core energy showed relatively high loadings in both PC1 and PC2.

In PCs three and four, properties related to specific substitution patterns were revealed. Notably, the coplanar PCBs and tetra-*ortho* PCBs had low scores in PC3. PCBs with high PC3 scores included 106, 116, 142, and 160. These congeners are more heavily substituted on one phenyl ring, compared to the more homogenous substitution patterns shown for those with negative PC3 scores. This separation appears to be due to specific dipole

moments in combination with spectral characteristics and the Erot. In conclusion, most of the physico-chemical variation in the 154 tetra- to hepta-chlorinated biphenyls was explained in the first two PCs, which were correlated to the number of chlorine atoms and the conformation of the congeners. The chemical domain of the PCBs are summarised in Figure 10 and the chemical variation is quantified in the principal properties or the score values for each congener.

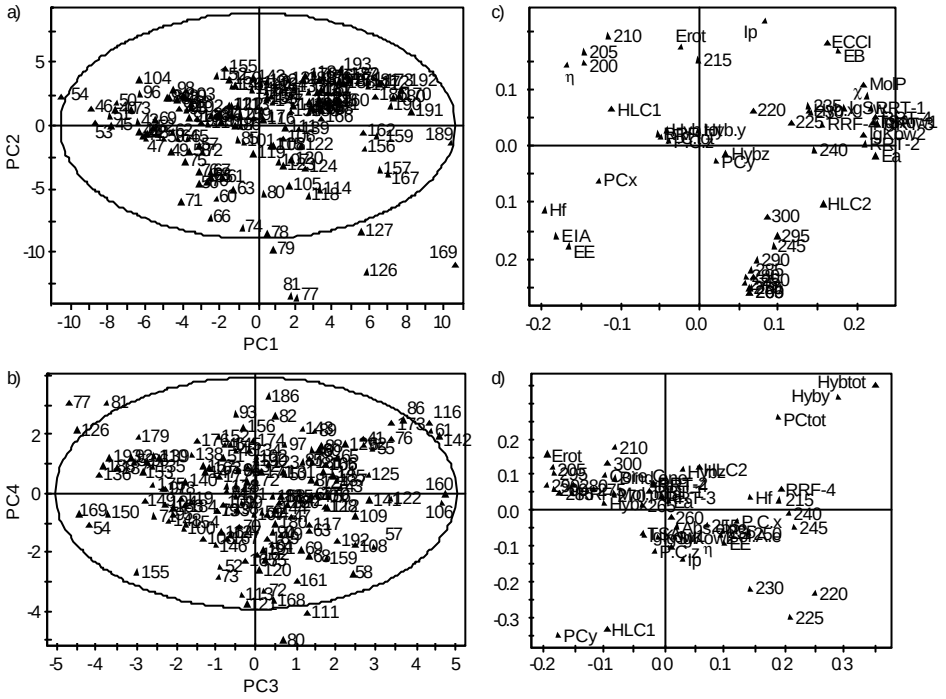

Figure 10. A PCA model including the 52 physico-chemical descriptors (Table 4) for the tetra- to hepta-chlorinated biphenyls. Figures a and c are the score and loading plots for principal components 1 and 2 (PC1-2) and Figures b and d the score and loading plots for principal components 3 and 4 (PC3-4). The Hotelling´s T^2 tolerance ellipse in the score plots was set to 95% tolerance (SIMCA).

6. Selection for screening and optimisation

The procedure for selecting compounds for training the QSAR model is crucial. The training set should include compounds that collectively cover the variation in physico-chemical properties of the studied class of chemicals. Further, the number of untested compounds that can be interpolated from the QSAR model is determined by the representativeness of the training set. The predictive capacity of the QSAR model can be validated by internal validation, e.g. cross-validation, or through an external validation set. In this thesis 20 PCBs were selected, according to a statistical experimental design for use as a training and validation set (Paper IV).

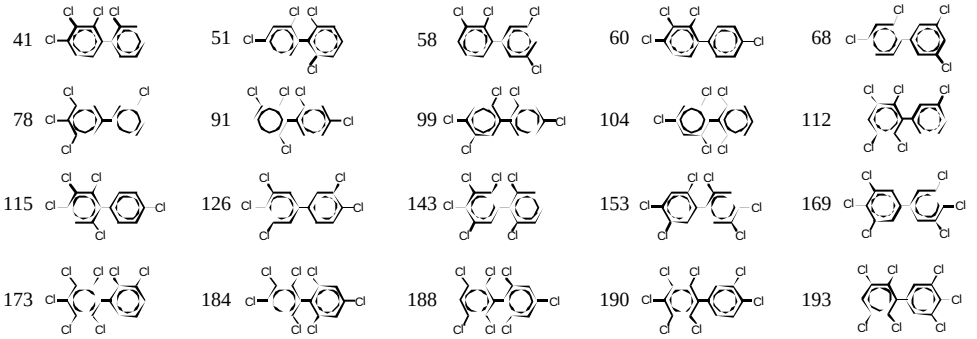

Figure 11. The molecular structures of the 20 PCBs selected to represent the tetra- to hepta-chlorinated biphenyls.

The principal properties of the compounds, calculated from a PCA including 47 physico-chemical parameters, were used as orthogonal descriptors of the chemical and structural variation. The first four PCs in the PCA explained 39%, 13%, 10%, and 7% of the total variation, respectively. The principal properties were applied in a 2^4-factorial design to generate 16 design levels. Two to fourteen PCBs were found on each design level, from which a few were selected to act as representatives of each level, see Table 5. In the final selection the most extreme congeners were avoided, except PCBs 153 and 169, which were included because of their abundance and toxicological potency, respectively. The 16 selected PCBs each represent a sub-space of the chemical domain. The molecular structures of these PCBs and four centre points, added to capture the interior parts of the domain, are displayed in Figure 11. The selected PCBs can be divided into a training and a validation set by using a 2^{4-1} fractional factorial design, see Table 5. These half fractions represent the whole class and can thus be used singly for

purposes such as pilot-studies, or in investigations for which the complete set would be too large for the testing protocol.

Table 5. The candidates and the final selection of the training and validation set on each design level of the 2^4 full factorial design (Paper IV). In addition, PCBs representing an Aroclor mixture (Tysklind *et al*. 1996) are listed, as well as those selected by D-optimal design.
[a]validation set congeners

Design levels					Candidates	Paper IV	Aroclor	D-optimal
1	+	+	+	+	173,174,181	173	146,183	41
2	-	+	+	+	46,51,89	51[a]	52,91,110	50
3	+	-	+	+	114,126,156	126[a]	151,199,201	54
4	-	-	+	+	55,60,81	60	44,45,49,101	71
5	+	+	-	+	147,175,188	188[a]	128,156,180,191, 194	77
6	-	+	-	+	54,103,104	104	66, 74	78
7	+	-	-	+	153,158,169	169	141,185	80
8	-	-	-	+	68,72,80	68[a]	33, 123	81
9	+	+	+	-	143,182,186	143[a]	149,168,178,193	82
10	-	+	+	-	41,45,88	41	26,27,31	106
11	+	-	+	-	180,190	190	179,197,202,209	110
12	-	-	+	-	67,76,78	78[a]	19,53	127
13	+	+	-	-	177,183,184	184	158,171,177,206	143
14	-	+	-	-	73,91,98	91[a]	22,28,42,118	155
15	+	-	-	-	162,191,193	193[a]	174,176,195,200	157
16	-	-	-	-	58,59,70	58	17,25,51,91	160
17	0	0	0	0		99	149	169
18	0	0	0	0		112[a]	151	170
19	0	0	0	0		115	168	192
20	0	0	0	0		153[a]		193

An additional set of PCBs has been selected to represent the chemical domain of the PCBs in a 1:1:1:1 mixture of Aroclors 1232, 1242, 1248, and 1260 (Tysklind *et al*. 1996). A PCA was calculated including 52 abundant PCBs from this mixture and 47 physico-chemical descriptors. A factorial design was applied, in combination with the principal properties of the compounds, as described above, to select congeners representing the chemical variation in the Aroclor mixtures, see Table 5. These PCBs may be used as indicator compounds in exercises such as environmental monitoring

or biological testing programmes. In addition, D-optimal designs has been evaluated and compared with factorial design for selecting representative compounds. A set of 20 PCBs was selected using D-optimal design (G-efficiency 78%), see Table 5. In this design, which had not any specific centre points, PCBs 41, 78, 143, 169, 193 were included, in common with the factorial design. The D-optimal design appears to span the chemical domain in an equally efficient way to the factorial design, but tends to include extreme congeners that were excluded from the design levels in the factorial design, see Figure 12.

The factorial design was constructed to cover the entire chemical domain of the tetra- to hepta-chlorinated biphenyls. Thus, measured biological activities can be screened for the entire class of compounds using the 20 selected congeners. In QSAR studies, the screening phase should be followed by a second phase in which the model is optimised. The second phase can be focused on a specific part of the chemical domain with high probable biological activity or of special interest, such as environmental abundance. In the study reported in Paper VI, the potency of the PCBs to induce CYP1A activity was screened using the 20 PCBs. QSARs were established and used to predict the response for untested congeners. PCBs 66, 70, 74, 107, 122, 124, 159, 162, 170, and 191 were suggested for future testing of CYP1A related activities. This set of PCBs was selected from the 41 PCBs ranked highest, according to their dioxin-like activity. These 10 PCBs have all been found in fly ash (Ballschmiter *et al*. 1987), all but three (124, 159, 162) have been found in Aroclor mixtures (Schulz *et al*. 1989), and all but four (66, 124, 159, 162) have been found in human milk (Safe *et al*. 1985). Further, two of these 10 PCBs, 66 and 74, were found by Wingfors *et al*. (2000) in human adipose tissue.

An additional set of PCBs was created in a study concerning competitive binding to the estrogen receptor (ER). These 10 compounds, i.e. 44, 45, 47, 84, 95, 149, 151, 178, 183, and 187 were selected using an approach designed for selecting compounds from a minor part of the chemical domain (Eriksson *et al*. 2000). In the directional modelling procedure, suggested by these authors, chemical and biological data are used simultaneously. A PLS model was calculated based on 52 physico-chemical descriptors and the preliminary results from the screening phase, including the binding affinities for PCBs 41, 51, 91, 104, 184, and 188. The PLS model with two significant components predicted 59 PCBs as members of the model. These PCBs, and all the congeners that account for more than 1% of any of the Aroclor

products 1016, 1242, 1254, or 1260 (Schulz *et al*. 1989) are marked in the score plot in Figure 13. As shown in the plot, most of the PCBs in the commercial mixtures do not have very similar physico-chemical properties to those predicted to be strong competitive binders to ER. Thus, a selection procedure that considers the environmental relevance will not capture the most potent congeners. However, the 10 selected PCBs were chosen not simply according to their binding affinity to the ER, predicted from the PLS model, but also according to their presence in commercial mixtures, human adipose tissues, human milk and biota. Two of the ten PCBs, viz. 44 and 47, were not members of the PLS model. Thus, they were included because of the similarity of their principal properties to the PCBs capable of competing for ER binding, together with their significant binding affinities, as extrapolated from the model. The 10 selected PCBs include representatives from the tetra- to hepta-homologue groups and are di- or tri-*ortho* substituted.

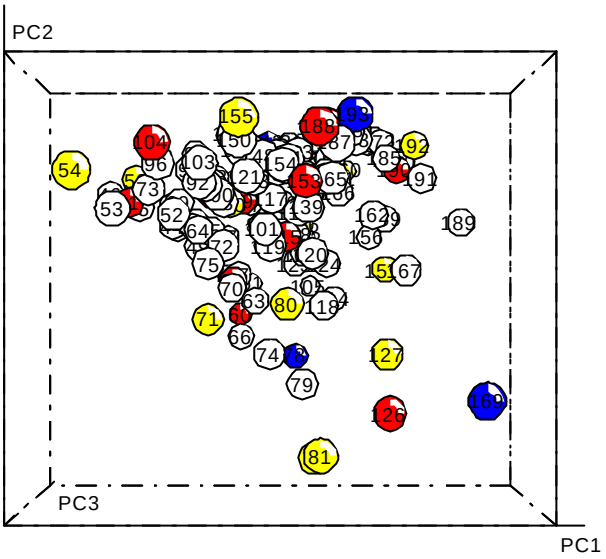

Figure 12. The chemical domain of the PCBs described by three principal components (PC1-3). The 20 PCBs selected using factorial design are marked in red, the D-optimal selection in yellow and those common to both are marked in blue.

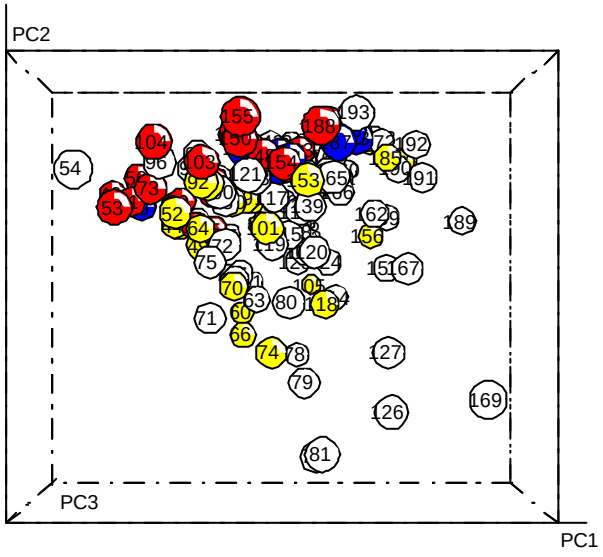

Figure 13. The chemical domain of the PCBs described by three principal components (PC1-3). The PCBs predicted to compete with 17β-estradiol for the rainbow trout estrogen receptor are marked in red, PCBs found at levels >1% in Aroclor mixture in yellow, and those PCBs that fulfil both criteria are marked in blue.

7. SAR and QSAR modelling

In general SAR and QSAR models are useful for increasing the knowledge of structure-activity relationships, for identifying outliers, for screening large groups of compounds, to set priorities for future research, and to facilitate risk assessment procedures. By applying the set of 20 selected PCBs in any test system, a broad range of chemical and structural variation is captured (Paper IV). In many test protocols some of the PCBs could be excluded prior to testing, especially if a very specific mechanism of action is being studied. However, by testing all 20 PCBs, the activity is screened for the studied classes of PCBs and both the responding and non-responding regions of the chemical domain can be identified. The activity of the compounds has to be determined in a qualitative or quantitative measure for the QSAR modelling procedure. In most cases the measured response is described by a single numerical value, such as the degradation percentage, biomagnification factor, LD_{50}, or EC_{50}. However, if the activity is measured over a complete dose-response curve, information may be lost by condensing the data into a single measurement. Ideally, therefore, the whole dose-response curve or a few key estimates should be used in QSAR models.

The SAR and QSAR models presented in this thesis include the 52 physico-chemical descriptors as the X-matrix and various biological activities as the Y-matrix. An overview of the environmental fate parameters and the biological activities measured for the 20 PCBs, or subsets of them, is summarised in Figure 14. The biological activities measured for the PCBs are described in 7.1. In the following section, QSARs are presented for selected responses induced by the 20 PCBs. These include the potency of the PCBs to inhibit intercellular communication, inhibit dopamine uptake, activate respiratory burst, compete for estrogen receptor binding and induce CYP1A related activities. Additional PCBs have been selected to improve the validity of the QSAR models, and the results from tests with these congeners are presented in 7.3. In section 7.4 predictions from the QSARs are discussed and the chemical domains of highly potent PCBs are identified. As shown in Figure 14, two measures of the physico-chemical stability of the 20 PCBs have been studied. In brief, the thermal degradation of the PCBs at 300 °C varied between 7 and 35%, but no clear structure-degradation pattern was found (van Bavel *et al*. 1996). Further, the rate of photolytic degradation of the PCBs varied between 0 to 6% per week, except for PCBs 60, 68 and 115, which degraded by between 11 and 25%

per week (Gidlund 1996). *Ortho* dechlorination was the major degradation pathway of the congeners.

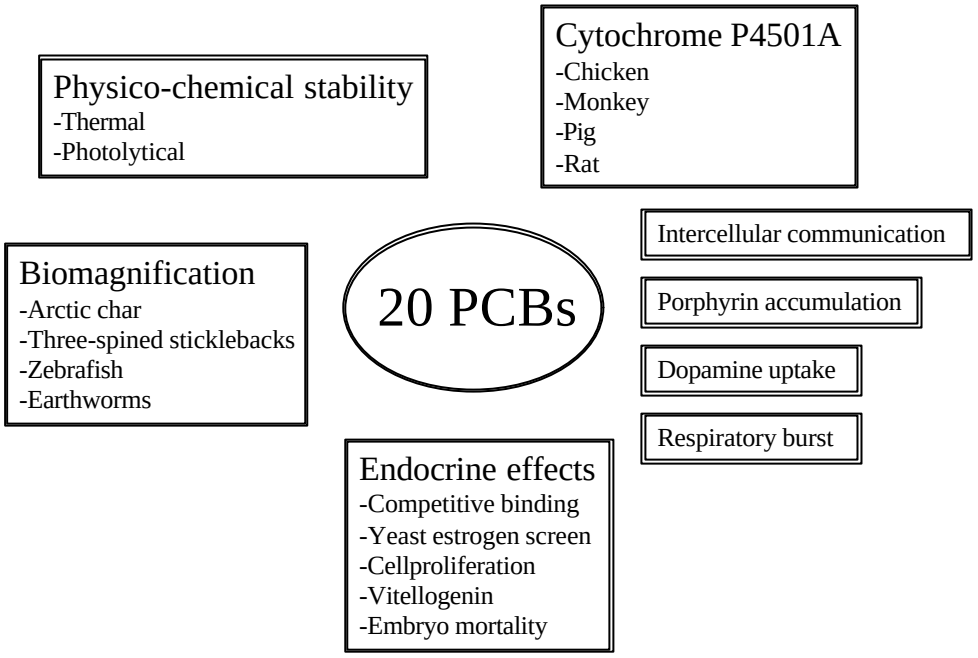

Figure 14. Summary of the environmental fate parameters and the biological responses measured for the 20 PCBs or sub-sets of these congeners.

7.1 QSAR modelling - the biological activities

The 20 PCBs, selected as described in Paper IV, have been applied in various biological test systems in collaboration with other research groups. The biomagnification factors and six additional biological responses of the PCBs are summarised in Table 6. A rough estimate of the PCBs potency in each system and their respective biomagnification factors in fish were scored from low (-) to high (+++) for each congener. Clearly, the studied responses varied widely among the PCBs, depending on their substitution patterns and the system investigated. Of the biological activities investigated, the inhibition of intercellular communication is discussed in Paper I, the biomagnification factors in Paper V and the PCBs potency to induce CYP1A related activities in Paper VI. In addition, various projects have been designed to study the endocrine activity of the PCBs using sub-sets of the 20 PCBs. Further, a large number of PCBs were found to be capable of

inducing accumulation of porphyrins in chicken embryo hepatocytes, see Table 6. Although the PCBs were capable to induce porphyrin accumulation, independent of their number of chlorine atoms and substitution patterns, the coplanar PCB 126 was the most potent followed by 169. These results, presented by Tysklind *et al.* (1995) will not be further discussed in this thesis.

Table 6. The 20 PCBs potency scored from low (-) to high (+++) for biomagnification in fish (BMF), inhibition of intercellular communication (GJIC), accumulation of porphyrins (PA), inhibition of dopamine uptake (DU), activation of respiratory burst (RB), binding to estrogen receptor (cER), and induction of CYP1A activity (CYP1A).

PCB	BMF	GJIC	PA	DU	RB	cER	CYP1A
41	–	++	++	+++	+	++	–
51	–	+++	++	++	+	++	–
58	++	–	+	++	+	+	–
60	++	+	++	++	+	+	++
68	++	–	+	++	+	+	–
78	–	–	+	+	–	–	++
91	++	++	++	+++	++	++	–
99	+++	–	+	++	+	+	+
104	–	++	++	++	+++	+++	–
112	++	++	+	+++	+	+	–
115	++	+	++	++	+	++	++
126	++	–	+++	–	–	–	+++
143	–	+++	+	+++	+	++	–
153	+++	++	++	++	–	+	+
169	++	–	+++	–	–	+	+++
173	+++	+++	–	++	–	++	++
184	–	+++	++	+	–	+++	–
188	++	++	+	+	–	+++	+
190	+++	+	–	++	–	–	++
193	+++	+	–	++	–	–	+

7.1.1 Biomagnification

Biomagnification is defined as the uptake of a compound via the food web that leads to an increase in concentration of the compound in the organism (van den Berg *et al.* 1995). The biomagnification factor (BMF) is determined by dividing the concentration of the compound in the organism at steady-state by the concentration of the compound in its food. The biomagnification factor of the compound is influenced by structure-specific

rates of uptake, and in addition also by the growth of the organism and losses through elimination, reproduction, and biotransformation. The 20 PCBs have been exposed through spiked food to zebrafish (*Danio rerio*) and three-spined sticklebacks (*Gasterosteus aculeatus*) (Paper V). Further, the uptake of the PCBs was examined in Arctic char (*Salvelinus alpinus*) and zebrafish after intraperitoneal (ip) injection of the compounds. Implantation of silastic capsules filled with the PCBs was tested in Arctic char as a third technique for exposing organisms to PCBs. In addition, earthworms (*Eisenia foetida*) have been exposed to the PCBs over a period of 31 days to study uptake and elimination kinetics (Wågman *et al*. 2000). Both the uptake and elimination were correlated with the molecular size of the compounds. The physico-chemical properties were most significant for the uptake during the first days of exposure. At steady state the significance of the SAR models decreased.

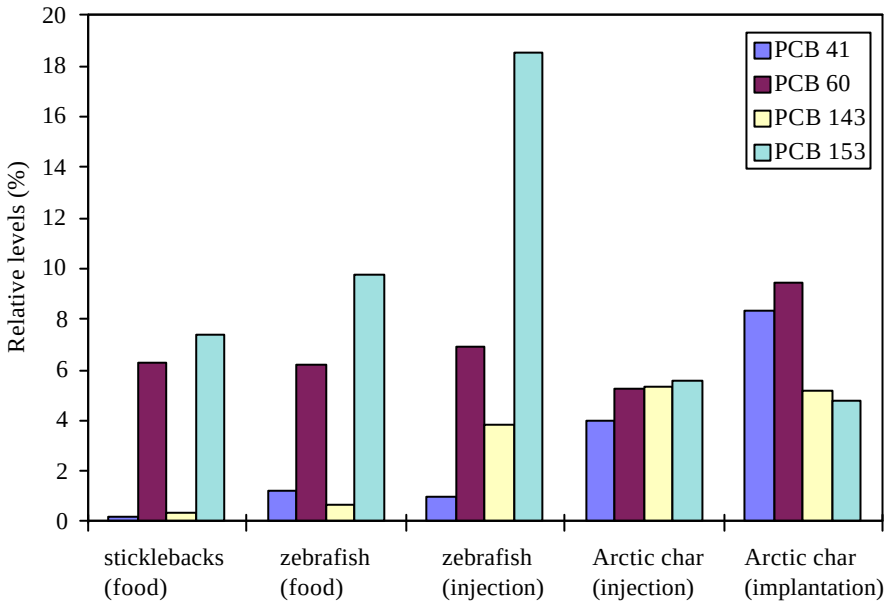

Figure 15. Relative levels of PCBs 41, 60, 143 and 153 after 35-140 days of exposure. Route of exposure is indicated in parenthesis. The levels were normalised relative to the levels in the food or peanut oil and to the sum of PCBs.

In Paper V, the uptake and elimination of the 20 PCBs were studied in relation to the substitution pattern of the compounds and the mode of exposure. Five different studies were discussed, viz. 1) dietary exposure in three-spined sticklebacks, 2) dietary exposure in zebrafish, 3) ip injection in

zebrafish, 4) ip injection in Arctic char, and 5) ip implantation in Arctic char. The results are summarised in Figure 15 by comparing the relative levels in the fish of four structurally characteristic congeners, i.e. 41, 60, 143, and 153. PCBs 41 and 143 have vicinal unsubstituted *meta-para* positions, whereas both 60 and 153 have chlorine atoms in both *para* positions, see Figure 11. In addition to structural differences, a large number of factors have to be considered when comparing the retention of these compounds in the fish. These include the ambient temperature, the carrier of the PCBs, the concentration of the PCBs, and the physiological status of the fish. The PCB pattern in the fish was most strongly altered (compared to the pattern in the source) in the three-spined sticklebacks and the zebrafish, see Figure 15. In the three studies concerning these species, PCBs 41 and 143 were found at lower relative levels compared to 60 and 153. This PCB retention pattern agrees well with the known structure-specific metabolic rates of the compounds. The PCBs with vicinal unsubstituted *meta-para* positions are known to be less strongly magnified in biota (Borlakoglu *et al*. 1993). In the Arctic char, the pattern of the PCBs was almost constant even after 70 days of exposure. The capacity to modify PCBs through biotransformation may be weaker in the Arctic char compared to the three-spined sticklebacks and the zebrafish. The concentrations of the PCBs retained in the Arctic char, exposed through the silastic implants, were inversely correlated with Kow. The most hydrophobic PCBs appear to remain in the capsules and thus the fish are most heavily exposed to the more hydrophilic compounds.

7.1.2 Intercellular communication

Gap junction intercellular communication (GJIC) is a specific mode of communication between cells, which is suggested to be associated with cell growth, differentiation, and maintenance of cellular homeostasis. Inhibition of the GJIC may be involved in carcinogenesis. Various PCBs, DDT, dieldrin, toxaphene, and brominated biphenyls have been shown to inhibit GJIC in human breast epithelial cells (Kang *et al*. 1996). Further, Kato *et al*. (1998) have shown that methyl sulfone PCBs are potent inhibitors of GJIC. The potential to inhibit GJIC has been measured for the 20 PCBs (unpublished results). The effects of the PCBs were examined at three concentrations (20, 30, 50 μM) in a scrape loading/dye transfer assay described by Hemming *et al*. (1991). These experiments were performed at the Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden. The potency of the compounds was ranked qualitatively from no

inhibition (0) to complete inhibition (3). Fourteen of the tested PCBs induced responses in the system over the whole dose-range tested. The most potent congeners were the tri- and tetra-*ortho* substituted PCBs, 51, 143, 173, and 184, see Table 6. In this assay, the coplanar PCBs 78, 126, and 169 produced no responses. Notably, the coplanar PCBs 77, 169 and 2,3,7,8-TCDD have previously been shown to inhibit GJIC in mouse Hepa1c1c7 cells (de Haan *et al.* 1994). Variations in the duration of exposure and type of cell line tested have been discussed as possible reasons for the inconsistency in SARs between the studies (de Haan *et al.* 1994).

7.1.3 Dopamine uptake

Neurochemical effects of PCBs have been observed both *in vitro* and *in vivo* (Brouwer *et al.* 1995). The PCBs have shown to affect the thyroid hormone system, which is critical in the perinatal development of the nervous system (Brouwer *et al.* 1999). Disturbed levels of these hormones can result in effects varying from subtle behavioural disturbances to gross mental retardation. Further, a very specific SAR was found for the decrease in dopamine content in rodent dopamine synthesising cells (Seegal *et al.* 1990; Shain *et al.* 1991). Among the 43 individual PCBs tested in this assay, those substituted in *ortho*- or *ortho-para* positions were the most potent. A common mechanism of action is suggested for the increase of Ca^{2+} influx and the translocation of protein kinase C (Brouwer *et al.* 1995). These effects were studied by Kodavanti *et al.* (1994, 1996) in rat cerebellar granule cells. The impact of PCBs on the uptake of dopamine in synaptic vesicles has been studied by Mariussen *et al.* (1999). The assay described by these authors was applied to study the SAR for inhibition of dopamine uptake. The effects of the 20 PCBs were examined in triplicate, at 15 μM, in 1999 at the Norwegian Defence Research Establishment, Kjeller, Norway (unpublished results). The potency of the PCBs was quantified as the mean dopamine uptake (in percentages) relative to the dopamine uptake in untreated control vesicles. The coplanar PCBs were non-inductive, and the *ortho*-substituted PCBs, such as 41, 91, 112, and 143 were the most potent, see Table 6.

7.1.4 Respiratory burst

PCBs have been shown to evoke diverse immunotoxic responses in exposed organisms (Holsapple 1996). The effects on the immune system may in part be related to a stimulation of intracellular Ca^{2+} levels in human granulocytes caused by *ortho* substituted PCBs (Voie and Fonnum 1998). *Ortho*

substituted PCBs have also been shown to activate a respiratory burst measured as luminol-amplified chemoluminescence in human granulocytes (Voie *et al*. 1998). These cells are of prime importance in the immune system, and long term exposure to PCBs may lead to an impairment of immunofunctions by inappropriate activation. The activation of respiratory burst was studied for the 20 PCBs plus 13 additional congeners (Voie *et al*. 2000). A chemo-luminescence assay was applied and the effects of all compounds at 20 μM were measured. Several of the PCBs had been found earlier to elicit a maximum response at this concentration. The height of the chemo-luminescence response curve normalised against the most active congener, viz. 104, was used as a measure of the response to the QSARs. The next most potent PCB after 104 was 91 followed by 51, 112 and 60, see Table 6. The three coplanar PCBs did not activate detectable respiratory burst in the human granulocytes.

7.1.5 Endocrine effects

The endocrine disrupting potency of the PCBs has recently been reviewed in a number of reports (Brouwer *et al*. 1999; Olsson *et al*. 1998). The PCBs have been shown to affect the estrogen and androgen system, the retinoid system, the thyroid hormone system, the corticosteroid system and several other endocrine systems (Brouwer *et al*. 1999). Various methods have been developed to study these effects and to screen potential endocrine disrupters (Ankley *et al*. 1998; Zacharewski *et al*. 1997). The 20 PCBs (or subsets of them) have been applied in collaborative projects for both *in vitro* and *in vivo* modelling of their estrogenic potency. The complete set was applied in a competitive binding assay and in a yeast estrogen screen assay. Further, subsets of the PCBs and selected OH-PCBs have been examined for their potency to induce cell proliferation, vitellogenin synthesis and embryo toxicity.

The binding affinity to various estrogen receptors was assessed for the 20 PCBs, a validation set of 10 PCBs, and 14 additional PCBs. The estrogenic potency of the PCBs was measured as their ability to compete with labelled (^{3}H) 17β-estradiol (^{3}H-E2) for binding to recombinant ERs from humans, green anole (*Anolis carolinensis*), and rainbow trout. The PCBs were tested in concentrations from 1 nM to 10 μM together with 2.5 nM ^{3}H-E2. The interaction of the PCBs to the ERs differed among the three species, which may be due to amino acid-sequence differences at the binding site (Matthews and Zacharewski 2000). In all assays PCBs 104, 184, and 188

competed significantly with ^{3}H-E2. IC_{50}-values between 0.4 and 1.3 μM were determined for these PCBs in the rainbow trout assay. This assay appeared to be the most sensitive, as PCBs 41, 51, 91, 115, 143, and 173 also competed to some extent with ^{3}H-E2 for binding to the rainbow trout ER, see Table 6.

None of the 20 PCBs were found to be capable of inducing estrogenic activity in the yeast (*Saccharomyces cerevisiae*) assay described by Routledge and Sumpter (1996). These experiments were performed at the Department of Pathology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden (unpublished results). The PCBs were tested in six concentrations (1 nM-1 μM) in ethanol with four replicates per concentration. To each plate, E2 was added as a positive control (1 pM-1 nM). The potency of the PCBs to inhibit the estrogenic activity was also studied. In these experiments, PCBs 51, 104, 112, 115, 126, 153, and 169 were co-administered with E2. However, the PCBs did not show any inhibitory effect on the estrogenic potency of E2, although all possible combinations of the previously tested concentrations were applied. Thus, in this assay the PCBs appear to be non-active as both estrogens and anti-estrogens.

The potency to induce vitellogenin synthesis in primary hepatocytes of rainbow trout, and cell proliferation in MCF-7 cells, was assayed for PCBs 58, 104, 112, 188 and five OH-PCBs (Andersson *et al*. 1999b). The OH-PCBs were selected to structurally match their parent compounds as well as possible, see Figure 16. The cell proliferation assay using MCF-7 cells has previously been described by Blom *et al*. (1998). The synthesis of vitellogenin in the rainbow trout hepatocytes was measured by an indirect ELISA method (Andersson *et al*. 1996). In both assays, the PCBs were tested in the concentration range 0.01 to 5 μM, with four to ten replicates. Further, the data were normalised to the positive control, E2, and the negative control with 0.1% ethanol in the medium. In the rainbow trout hepatocytes, OH-PCBs 4'-30, 4'-50, 4'-112, and 4'-72 (see Figure 16) significantly induced vitellogenin synthesis at one or more concentrations. Proliferation of the MCF-7 cells was significantly enhanced by all OH-PCBs. However, the most potent OH-PCBs were those substituted with chlorine atoms in the 2,4,6-positions and a hydroxy-group with adjacent hydrogen atoms on the opposite phenyl ring, i.e. 4'-30 and 4'-50. In common with the competitive binding assay, PCBs 104 and 188 showed estrogenic potency in the MCF-7 cells. The results show that these PCBs were capable to bind to

the ER and induce an ER mediated response. These congeners are also substituted in the 2,4,6-positions, confirming the significance of this substitution pattern. However, none of the PCBs induced responses in the rainbow trout assay over the range of tested concentrations. To summarise, the study clearly showed that the estrogenic potency of the hydroxylated PCBs was higher than that of the corresponding PCBs.

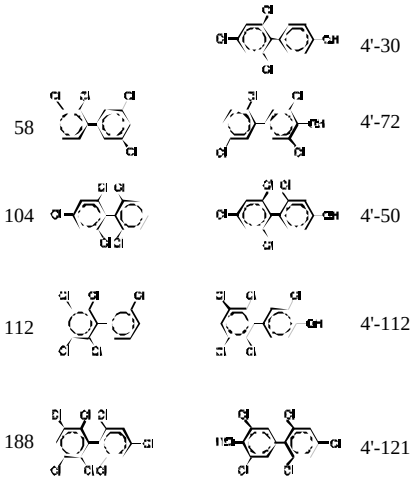

Figure 16. Molecular structures of PCBs 58, 104, 112, 188 and OH-PCBs 2',4',6'-trichloro-4-biphenylol (4'-30), 2',3,5,5'-tetrachloro-4-biphenylol (4'-72), 2,2',4',6'-tetrachloro-4-biphenylol (4'-50), 2',3,3',5',6'-pentachloro-4-biphenylol (4'-112), and 2',3,4',5,6'-pentachloro-4-biphenylol (4'-121).

The same four PCBs (58, 104, 112, 188) were studied *in vivo* in Atlantic salmon by repeatedly injecting the compounds dissolved in peanut oil (Norrgren *et al.* 1999). Fourteen to seventeen days after the first injection, blood was sampled, the vitellogenin was measured, and compared with the negative control (peanut oil) and positive control (E2). None of the PCBs induced vitellogenin synthesis in the Atlantic salmon at any of the concentrations tested (0.8 and 80 mg/kg body weight). A mixture of the 20 PCBs in equal concentrations administered to zebrafish through spiked food has been shown to cause reproductive disturbances in the fish (Örn *et al.* 1998). The median survival time of the offspring of the exposed females was reduced in the high-dose group, and egg production was reduced in all three dose groups studied. Further, PCBs 60, 104, OH-PCB 4'-30, and E2 were highly toxic to embryos following maternal exposure and transfer to the oocytes of zebrafish (Westerlund *et al.* 2000). In this study, eight of the 20 PCBs, viz. 60, 104, 112, 126, 143, 173, 184, and 190, and two OH-PCBs

(4'-30 and 2',3',4',5'-tetrachloro-3-biphenylol) were injected into zebrafish (1 μmol/kg body weight). A correlation between early life stage mortality and estrogenic potency was suggested as the highly embryotoxic PCBs 104 and 4'-30 were shown to induce expression of the hepatic ER mRNA in the male zebrafish (Westerlund *et al*. 2000). However, the study indicated that this was due to a different mode of action as PCB 60 was embryotoxic but did not induce ER mRNA.

7.1.6 CYP1A related activities

The cytochrome P450 enzymes are heme proteins that play an important role in the biotransformation of fatty acids, steroids, drugs and vitamins, as well as xenobiotics (van Holde and Mathews 1990). These enzymes are involved in the phase I conversion of the parent compound into a more hydrophilic form. Some of the CYP enzymes can be induced by their substrates, in an adaptive response to a changing chemical environment (Whitlock 1999). The PCBs have been shown to induce diverse levels of expression of the CYP1A and CYP2B gene families (Safe 1994). CYP1A1 induction is initiated by the binding of the inducer to the cytosolic Ah-receptor following transport of a transformed ligand-receptor complex to the nucleus (Rowlands and Gustafsson 1997). Interaction of the complex with specific DNA sequences, such as the xenobiotic responsive element and the Ah-receptor responsive element, activates transcription of the CYP1A1 gene. In turn this activation leads to increased mRNA levels, followed by an increase in CYP1A1 related activities (Rowlands and Gustafsson 1997). The induction of the hepatic cytochrome P450 monooxygenases is a sensitive indicator of exposure to xenobiotics, such as PCBs, in fish, mammals and birds (Stegeman *et al*. 1992). Their induction is frequently evaluated by measuring dealkylating activities, such as ethoxy-resorufin-*O*-deethylase activity or methoxyresorufin-*O*-demethylase activity (MROD) (Nerurkar *et al*. 1993; Burke *et al*. 1994). Further, relative effect potencies (REPs) of PCBs are often based on EROD activities measured *in vitro* in hepatocytes from various organisms including rat (Schmitz *et al*. 1995), rainbow trout (Clemons *et al*. 1996), chicken (Kennedy *et al*. 1996), pig (van der Burght *et al*. 2000), and monkey (van der Burght *et al*. 1999).

In Paper VI, QSAR models were established for the PCBs potency to induce EROD and MROD activity in primary hepatocytes from cynomolgus monkeys, castrated male pigs, and chicken embryos. The response data were generated and presented by van der Burght *et al*. (1997, 1999, 2000). MROD

activity was measured by the chicken assay and both EROD and MROD by the monkey and pig assays. EROD activity, for all 20 PCBs, has previously been assayed in chicken hepatocytes (Tysklind *et al*. 1995). The hepatocytes were exposed to the 20 PCBs in triplicate, and in 12 concentrations from pM to μM. The dealkylating activities were estimated as EC_{10}, EC_{50}, EC_{90}, and maximal response relative to PCB 169 (Ymax). CYP1A induction in one or more of the assays was observed after exposure to 12 of the 20 tested PCBs and the most potent were found to be the non-*ortho* PCBs followed by the mono-*ortho* PCBs, see Table 6.

The EROD activity induced by the 20 PCBs was recently measured in rat hepatoma MH1C1 cells (unpublished results). The experiments were performed at the Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden using an assay basically described by Hanberg *et al*. (1991) and Kennedy *et al*. (1993). The PCBs were tested in seven concentrations from 0.01 to 25 μM on 96-well plates with six wells per concentration. Preliminary results indicate that all of the 20 PCBs, except congeners 51, 91, 99, 104, and 184, induced EROD activity at the highest tested concentration. The most potent PCB was 126, followed by 169, 193, 188, 78, 112, 143, and 58, see Table 7. Most of the PCBs induced only a low response in this system, with little variation. Unexpectedly, the coplanar PCB 78 showed lower or equal induction potency compared to various *ortho* substituted congeners. The EROD-inducing potency of the various PCBs was less varied in the rat hepatoma cells compared to the results from the chicken, monkey and pig assays.

Table 7. Relative effect potencies (REPs) calculated relative to 2,3,7,8-TCDD and the maximal response (Ymax) relative to PCB 169 for the 20 PCBs in the rat hepatoma MH1C1 cells.

PCB	REP	Ymax	PCB	REP	Ymax
41	-	-	115	-	-
51	-	-	126	0.09	110
58	$1.9*10^{-6}$	95	143	$2.0*10^{-6}$	24
60	-	-	153	-	-
68	-	-	169	$3.3*10^{-3}$	100
78	$3.8*10^{-6}$	46	173	-	-
91	-	-	184	-	-
99	-	-	188	$5.2*10^{-6}$	123
104	-	-	190	-	-
112	$2.0*10^{-6}$	25	193	$1.1*10^{-5}$	77

7.2 QSAR modelling - the screening phase

The 20 PCBs were selected in the study described in Paper IV to represent the 154 tetra- to hepta-chlorinated biphenyls. By testing these congeners, QSARs can be established to predict the potency of untested congeners from the entire class of compounds covered. The chemical variation of these groups of homologues can thus be screened and sub-parts of the chemical domain can be identified for more detailed studies. Below, QSARs are described for five selected biological responses.

7.2.1 Intercellular communication

In Paper I a QSAR model was calculated based on the data presented by Hemming *et al*. (1991) for 27 PCBs. These PCBs were measured at six concentrations (6.25-100 μM) using a scrape loading/dye transfer assay to yield a qualitative response to the compounds. The highest concentration at which there was no inhibition of dye transfer, and the lowest concentration at which the inhibition of dye transfer was complete, were reported (Hemming *et al*. 1991). For QSAR modelling the concentration of PCBs causing 50% inhibition (IC_{50}) of GJIC was calculated by combining the highest concentration giving no inhibition, and the lowest giving complete inhibition. In addition, the effects of the 20 PCBs have been examined at three concentrations in the same assay. To study the SAR for PCBs potency to induce GJIC, and to validate different response parameters, various PLS models were calculated, see Table 8. According to explained variance and cross-validated explained variance, the PLS model including the IC_{50} values from Hemming *et al*. (1991) was the most significant. Descriptors of significance in this model were those correlated with *ortho* substitution, such as the absolute hardness, the κ-band from the UV-spectra, and the electron affinity. The PLS models calculated with no and complete inhibition (as defined above) as y-variables showed less predictive capacity, see Table 8.

The PLS models including the data from the 20 PCBs were less significant in terms of Q^2 values, see Table 8. However, similar SARs were observed and essentially the same physico-chemical descriptors were significant in these models. The models with the highest predictive capacity were those including the response at 50 μM and the IC_{50} values. In comparison with the data presented by Hemming *et al*. (1991), the dose-response curves were less fully covered for the 20 PCBs as only three concentrations were tested. In addition, the tested congeners from both studies appear to cover the chemical domain of the PCBs in an equally efficient way.

Table 8. Explained variance in the X-matrix (R^2X), the Y-matrix (R^2Y) and the cross-validated explained variance (Q^2) from various PLS models of PCBs potency to inhibit intercellular communication. The models include one significant component and are based on data from Hemming *et al*. (1991) (models 1-4) and data from the 20 PCBs (models 5-9).

	Response	R^2X	R^2Y	Q^2
1	No Inh	37	75	60
2	Compl Inh	38	74	68
3	No + Compl	37	73	63
4	IC_{50}	38	80	72
5	20	0	0	0
6	30	33	40	26
7	50	31	62	54
8	20, 30, 50	31	37	28
9	IC_{50}	31	61	53

7.2.2 Dopamine uptake

PLS models were calculated including the mean uptake of dopamine into synaptic vesicles. The first PLS model ($R^2Y=0.58$, $Q^2=0.45$) included the responses induced by the 20 PCBs in addition to PCBs 49, 54, 77 and 103. This model revealed outlying behaviour of PCB 54, and very low activities for the coplanar PCBs. A high potential to disturb the dopamine uptake was shown for the di- and tri-*ortho* PCBs. The Q^2 value increased from 0.45 to 0.73 by excluding 54 from the model. Thus, the model was not trained to handle the inactivity of the fully *ortho* substituted 54. Significant physico-chemical descriptors in this model were the κ-band from the UV-spectra, the absolute hardness and the ionisation potential. These descriptors were correlated with the number of chlorine atoms in the *ortho* positions, but also to the size of the molecule, see Figure 6. The internal barrier of rotation, which is particularly strongly *ortho* correlated, was of moderate importance in this model.

In order to study the SAR for medium and high potency congeners in more detail, a third PLS model was calculated, including the tetra- to hepta-chlorinated biphenyls, except PCB 54 and the coplanar PCBs. The statistical significance of this model was weak ($R^2Y=0.34$, $Q^2=0.17$). The most active congeners were *meta* substituted, di- and tri-*ortho* substituted with less than seven chlorine atoms. Additional experiments are warranted, including the complete dose-response curves to complement this screening of the potency of the PCBs to inhibit uptake of dopamine into synaptic vesicles. A very

specific mechanism of action may be involved, since neither the number of chlorine atoms nor the degree of *ortho* substitution appears to be crucial.

7.2.3 Respiratory burst

A PLS model including the potency to activate respiratory burst in human granulocytes for the 20 PCBs showed an explained variance of 0.65 and a cross-validated explained variance of 0.48 with one significant component. The correlation between the observed relative responses and those predicted by the model are shown in Figure 17. Six additional tetra- and penta-chlorinated biphenyls, viz. 46, 47, 49, 50, 54, and 103 were measured in this assay. These PCBs were used for external validation of the model, although they were not selected as representatives for the studied class of PCBs. A RMSEP of 28% was calculated, based on these six PCBs. The poor representativeness of the external validation set can be seen in Figure 17, in the incomplete spread of the PCBs over the range of potencies. The size of the molecule and the number of *ortho* substituents were found to be important factors related to the PCBs potency to activate respiratory burst. In a second PLS model, the 13 PCBs with a chemoluminescence response above 30% were included. However, this model was not able to capture the SAR of this response, and no significant components were found. PCBs 46, 51 and 54 were predicted to be more potent than actually observed in the assay. These PCBs are tri- and tetra-*ortho* substituted, but lack the 2, 4, 6-substitution pattern that is shared by the two most active PCBs. This pattern is, however, found in PCB 103, which only showed a height of 27%. The activation mechanism of the respiratory burst in human granulocytes may involve at least two different intracellular pathways (Voie *et al*. 2000). The physico-chemical prerequisites for a potent activator appear to be multivariate, and include moderate size, a high internal barrier of rotation, and *para*-substitution.

7.2.4 Competitive binding to ER

In order to study the SAR of the PCBs potency to compete with E2 for binding to rainbow trout recombinant ER, a PLS model was calculated. The potency of the PCBs was ranked from zero to three, as suggested by Matthews and Zacharewski (2000), i.e. according to whether they were defined as strong binders, medium binders, weak binders or non binders. Among the 20 PCBs, three were classified as strong binders, six as medium binders, seven as weak binders and four as non binders. The estrogenic

potency of the PCBs was correlated with the number of *ortho* chlorine atoms. The most potent PCBs were the tetra-*ortho* substituted, while the coplanar PCBs were either non binders or weak binders (169). The PLS model with three significant components explained 90% of the variation in the ER binding potency and 69% of the cross-validated variance. The internal barrier of rotation, Henry's law constant, ionisation potential and the κ-band from the UV-spectra were significant descriptors in the model.

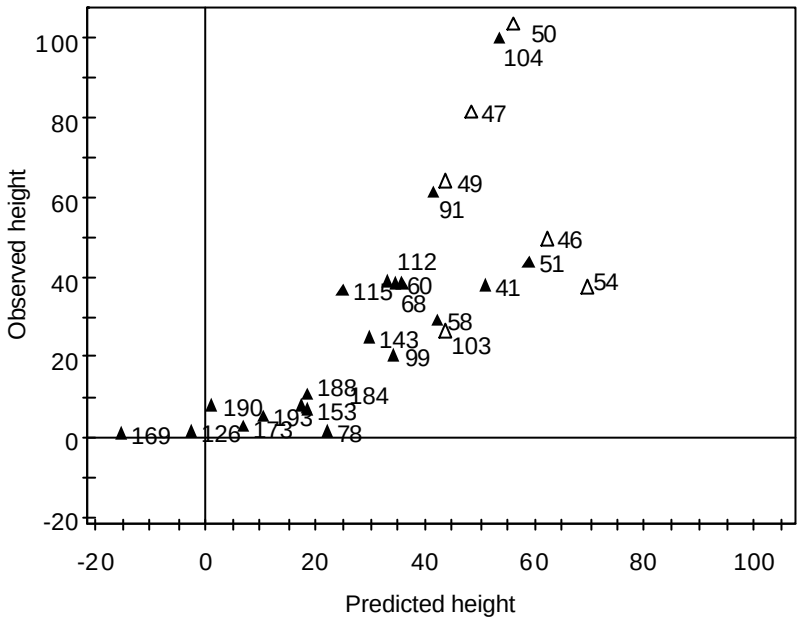

Figure 17. Observed versus predicted potencies for activation of respiratory burst in human granulocytes by various PCBs. The potency of the PCBs is given relative to the strength of response to PCB 104 and the training set is marked with filled triangles and the validation set with open triangles.

7.2.5 CYP1A related activities

The potential of the PCBs to induce EROD and MROD activity in primary hepatocytes from chicken, monkey and pig was discussed in Paper VI. Initially, four different measurements of the dose-response curve, viz. EC_{10}, EC_{50}, EC_{90} and Ymax were treated simultaneously in the PLS models. However, since higher Q^2 values were achieved with the single response models, EC_{50} and Ymax were applied individually as the dependent variable for the final QSARs. To optimise the precision in the predictions of untested congeners, a two-step modelling procedure was applied in Paper VI. The

first model included all 20 tested congeners and was constructed to distinguish between PCBs with and without activity. The non-active PCBs were assigned an EC_{50} value one order of magnitude greater than the highest tested concentration. The final REPs were calculated based on the predictions from a second model established only with the active inducers. Congeners assigned REPs were limited to those with an EC_{50} value below a limit set three orders of magnitude greater than the value of the most potent PCB, and a Ymax value greater than 50% relative to PCB 169.

Since the CYP1A induction potency of the 20 PCBs was measured in hepatocytes from three different species and two dealkylation activities were assayed, species- and isoenzyme-specific differences were studied. Two procedures were applied to compare these results, viz. a simple comparison of the predicted EC_{50} values, and a PLS modelling approach including all measurements from each assay. In the first procedure, the predicted responses were plotted and the regression equations and coefficients were compared. Generally, the CYP1A related activity was correlated between the species, but the sensitivity of the species differed in relation to exposure to different structural classes of PCBs. In summary, the chicken assay showed the most specific SAR, with high activity for the non-*ortho* PCBs. The SAR of the pig assay was the most general, as even di- to tetra-*ortho* PCBs were predicted to be inducers. EROD and MROD responses were compared by calculating PLS models, where $EC_{10,50,90}$ and Ymax values from the EROD assay were assigned as the X-matrix and the corresponding parameters of the MROD assay were assigned as the Y-matrix. Thus, the complete dose-response curves were compared. A comparison of the Q^2 values between the monkey and the pig models showed that the EROD and MROD responses were more strongly correlated in the monkey (0.94) than in the pig assay (0.82). This may indicate that the same isoenzyme of CYP1A is involved in the measured dealkylating activity, at least in the monkey hepatocytes.

To encompass all measured CYP1A associated activities from the chicken, monkey and pig assays in one measure, the principal induction potency (PIP) of the PCBs was calculated. Initially, a PCA was calculated including all 20 response parameters for the 20 tested PCBs. This model explained 85% of the variation by one significant component. The score values of the 20 PCBs from this PCA, i.e. the principal properties or the principal induction potencies, were applied in a PLS model to predict the PIPs of untested congeners. The model with two significant components explained 82% of

the cross-validated variance, and the principal induction potencies were predicted for all 154 tetra- to hepta-chlorinated biphenyls. The final PIPs were calculated relative to PCB 126, which was assigned the PIP value 1. These values summarise the CYP1A induction potency of the PCBs over three species, and could be used as a tool in risk assessments of these compounds, and to identify PCBs for future testing of Ah-receptor related activities (Paper VI).

7.3 QSAR modelling - the optimisation phase

The QSARs established for the 20 PCBs in the screening phase should be refined in a second phase, the optimisation phase, involving the biological testing of compounds selected from the chemical domain of highest probable activity, or those of special interest. This is warranted, in particular, if biological activities are studied with very specific mechanisms of action. The model can be optimised to predict the most active compounds with higher accuracy or to increase the resolution in the model for the chemical domain between the weakly and highly active PCBs. In this project, the second approach was adopted for assessing the potency of the PCBs to compete for ER binding and to induce CYP1A related activities. As described in chapter 6, additional sets of PCBs were selected based on their predicted biological activity as well as their environmental abundance. The results for the validation of QSARs including these PCBs are summarised below, for endocrine and CYP1A related activities.

The 10 PCBs selected for optimising the model concerning competitive binding to recombinant ERs, viz. 44, 45, 47, 84, 95, 149, 151, 178, 183, and 187 did not induce responses in the anole- and human-ER assays. However, in the rainbow trout assay PCBs 45 and 47 displaced 50-70% of the ^{3}H-E2 from the recombinant ER and the remaining eight PCBs were characterised as weak binders (<30%). Further, these congeners were used for external validation of the QSAR model established with the 20 PCBs. In this model most of the 10 PCBs were miss-classified into too high classes, see Figure 18. The non-potent and the most potent congeners were predicted reasonably well, in comparison with the moderate and weak binders. The model is probably weak due to the relatively low precision of the potency assessments, based on the constructed ranking scale. However, among the set of environmentally relevant PCBs two (45 and 47) were found to be capable of competing with 17β-estradiol for binding to the recombinant

rainbow trout ER. Further, by testing these congeners, the SAR for the PCBs potential to compete for ER binding was refined.

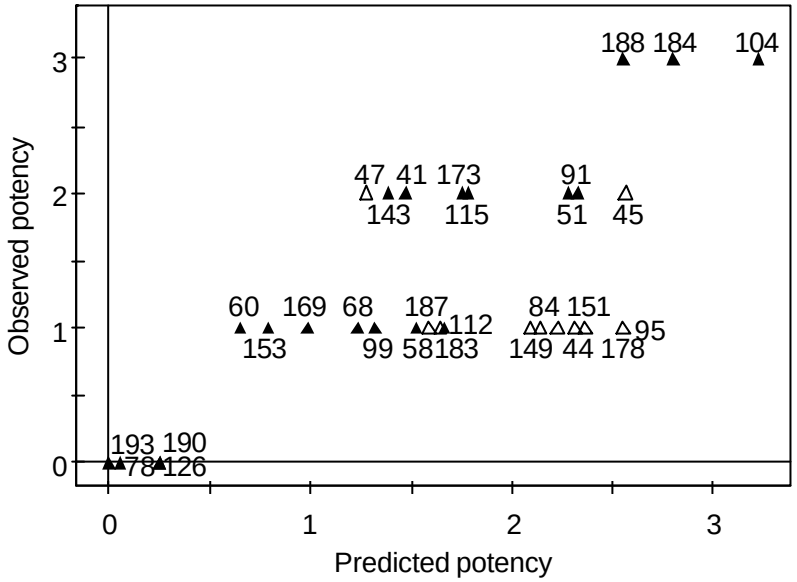

Figure 18. Observed versus predicted potencies of PCBs to compete with 17β-estradiol for binding to rainbow trout recombinant ER. The training set is marked by filled triangles and the validation set by open triangles.

To validate the PIPs, calculated from the CYP1A related activities measured in hepatocytes from chicken, monkey and pig, PCBs 66, 70, 74, 107, 122, 124, 159, 162, 170, and 191 were selected (Paper VI). These were predicted to be moderate or weak inducers according to their PIP values. The potency of the 10 PCBs has recently been measured in a rat hepatoma cell assay. Preliminary results indicate that all 10 PCBs were weak inducers of EROD activity in the rat hepatoma cells, see Figure 19. None of the PCBs reached the maximal response as compared to 2,3,7,8-TCDD. PCBs 74, 124, and 191 were the most potent and their REPs calculated relative to 2,3,7,8-TCDD were between $0.8*10^{-6}$ and $1.2*10^{-6}$. The maximal responses for PCBs 70, 107, 122, and 162 were 30 to 40% compared to 2,3,7,8-TCDD. In agreement with their PIPs, most of these PCBs were capable of inducing EROD activity. However, to validate the PIP ranking scale, further testing of these PCBs is warranted, including the three original test systems.

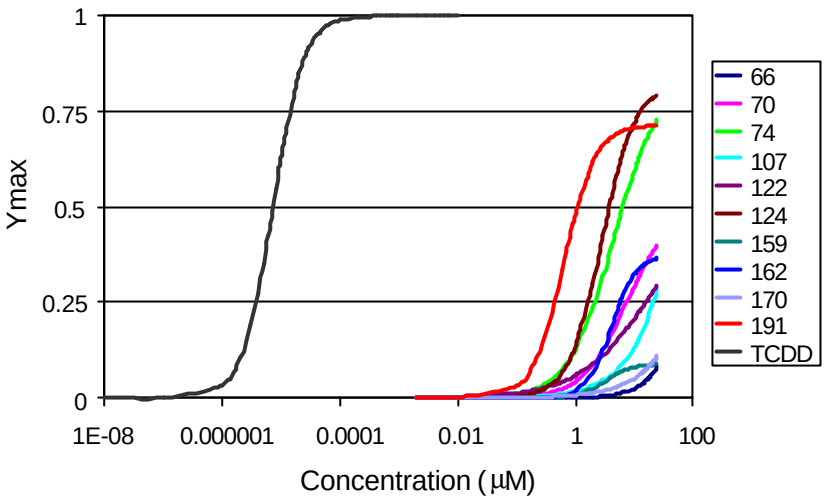

Figure 19. Induction of EROD activity in the rat hepatoma MH1C1 cells after treatment with PCBs 66, 70, 74, 107, 122, 124, 159, 162, 170, 191, and 2,3,7,8-TCDD.

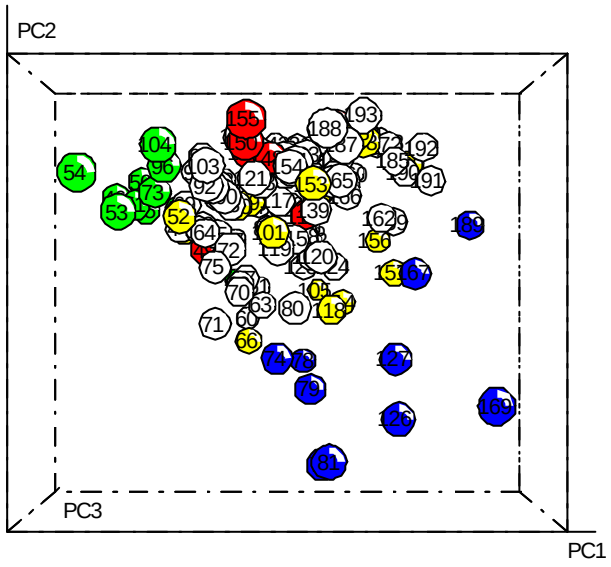

Figure 20. The chemical domain of the PCBs described by three principal components (PC1-3). PCBs predicted as the most potent to disturb intercellular communication, dopamine uptake and respiratory burst are marked in green, PCBs predicted to compete with 17β-estradiol for the rainbow trout recombinant estrogen receptor in red, PCBs quantified in human blood and adipose tissue from a Swedish general population in yellow and PCBs predicted to be CYP1A inducers are marked in blue.

7.4 Summary

QSARs have been established for the PCBs potency to inhibit gap junction intercellular communication, inhibit dopamine uptake in synaptic vesicles, activate respiratory burst in human granulocytes, compete with 17β-estradiol for binding to rainbow trout recombinant ER and to induce CYP1A activity. To summarise these models, the 10 PCBs predicted to be most active in each assay were considered. These PCBs were marked in a score plot including principal components one to three, see Figure 20. By this procedure, the chemical domain was identified of the most potent congeners for each biological response. In addition, congeners found in human blood and adipose tissue from a Swedish general population were marked (in yellow in Figure 20) to identify environmentally relevant PCBs (Wingfors *et al*. 2000). As well as the PCBs quantified by these authors, McFarland and Clarke (1989) suggested ten additional PCBs to be environmentally important, viz. 49, 70, 81, 87, 119, 123, 151, 158, and 168 (Table 2). However, these congeners have similar principal properties to those found in human blood and tissues, and are thus found in the same chemical domain as the PCBs marked in yellow in Figure 20.

The chemical domains of the PCBs with high potential for disturbing GJIC, dopamine uptake and respiratory bursts clearly overlapped, and hence the PCBs predicted to be potent in these assays were marked with a single colour (green). Generally, these PCBs are tetra- or penta-chlorinated and tri- or tetra- *ortho* substituted. The most potent congeners in these assays include 40, 43, 45, 46, 50, 53, and 96. Among these PCBs, 40, 45, 46, and 53 each comprise more than 0.5% of any Aroclor mixture (Schulz *et al*. 1989), Further, all but 40 and 43 have been found in fly ash from municipal waste incineration (Ballschmiter *et al*. 1987). However, the most potent inducers in these systems are not likely to be found in human tissues, see Figure 20. This also applies to PCBs predicted to compete with estradiol for binding to ER. These PCBs with strong ER-binding activity are also multi-*ortho* substituted, but in comparison to the PCBs listed above with high levels of other biological activities, they are more heavily chlorinated, see Figure 20. Examples of PCBs from this part of the chemical domain are 111, 136, 148, 150, 155, 176, and 179, of which 136, 176, and 179 have been found in Aroclor mixtures.

The PIP ranking scale was calculated as an interspecies measure to assess the PCBs potency to induce CYP1A related activities (Paper VI). In

common with the PCBs with high PIP values (blue spheres in Figure 20) is a substitution pattern with at least one *para* chlorine atom and less than two *ortho* substituents. Further, the PCBs assigned TEFs, see Table 3, were found amongst the 25 congeners with the highest PIP scores. Generally, these congeners are not abundant in Aroclor mixtures, but some of them have been found in human tissues and in fly ash, including 74, 167, 169, and 189.

The established QSARs indicate that most of the environmentally relevant PCBs will show low potency in any of the concerned bioassays. In agreement with this conclusion, the PCBs tested in the QSAR optimisation procedure, which were selected according to both their environmental relevance and their predicted biological potency, were found to have low or moderate activity. Further, the PCBs with the highest biomagnification factors in fish were those substituted in the *meta-para* positions and at least one *ortho* position (Paper V). The chemical domain of these PCBs coincides well with that of the PCBs found in human tissues. These congeners, as marked in yellow in Figure 20, are found in-between the chemical domains of the most potent PCBs. However, results of the studies presented in this thesis emphasise that in addition to congeners that possess Ah-receptor mediated toxicity, other structural subclasses of the PCBs are of concern. Thus, additional studies with PCBs covering chemical domains indicative of highly active compounds are warranted.

8. Concluding remarks and future perspectives

In this thesis a multi-step procedure has been applied to develop QSARs for the polychlorinated biphenyls. The tetra- to hepta-chlorinated congeners have been of particular interest since they were considered to be the most relevant due to their environmental abundance, persistence and toxicity. In Paper I, 52 physico-chemical descriptors were captured from the literature, measured and calculated. These descriptors were then used to model the PCBs potency to inhibit intercellular communication. Among the calculated semi-empirical descriptors, the ionisation potential, electron affinity and a descriptor combining these factors, viz. the absolute hardness, were frequently highly weighted in the PLS models. These descriptors reflect characteristics correlated to the size of the compounds in combination with their conformation. In contrast, the internal barrier of rotation is merely a descriptor of *ortho* substitution. However, as shown in Paper II this parameter also describes the number of buttressing *meta* substituents.

The UV-spectra measured for all 209 PCBs were shown to include detailed information on structure-specific characteristics of the compounds. These data have frequently been found useful in the QSAR models, especially as the UV-spectra reflect characteristics correlated with the compounds ability to adopt a coplanar conformation. In addition, the *para* substituents influence the UV-spectra of the PCBs, and chlorine atoms in these positions have proved to be crucial in particular for CYP1A-related activities. For interpretation of UV-spectra, PCA was shown in Paper III to be a useful tool.

To cover the class of chemicals studied in the QSARs, appropriate selection of training compounds is crucial. In Paper IV factorial design was applied, with the principal properties of the compounds as design variables, to select 20 PCBs. These compounds represent the dominant physico-chemical variation in the tetra- to hepta-chlorinated biphenyls. However, it is suggested that the initial screening of the studied biological effects should be followed by optimisation of the QSAR model. In Paper VI and chapter 6, two additional sets of PCBs were presented for analysis of CYP1A related and estrogenic responses. These congeners were selected from a limited region of the chemical domain after the screening covering the major physico-chemical variation. This phase in the QSAR procedure is particularly important if biological activities are studied that have very specific mechanisms of action.

The potency of the 20 selected PCBs has been assessed in various bioassays, along with their tendency to be biomagnified in fish. The BMFs of the PCBs were shown in Paper V to be influenced by species differences and to be strongly correlated with their substitution patterns. Each biochemical response measured was found to be specific and, consequently, the most potent congeners were found in a distinct part of the chemical domain. However, QSARs were established for all responses covered, since at least ten of the tested PCBs responded in most of the bioassays. Generally, the models developed in this project have been shown to be capable of predicting the potency of a large number of untested congeners. In most PLS models only ten to twenty PCBs were classified as non-members.

The predicted potencies of the PCBs can be used to prioritise future research and in risk assessments of this class of compounds. The PIP ranking scores presented in Paper VI are designed for such purposes and, especially, to assess the potency of the PCBs to induce Ah-receptor mediated responses. Further, by comparing the structural variation among the most environ-mentally abundant PCBs and those predicted to be biologically active, the validity of the responses can be evaluated. The results presented in this thesis indicate that PCBs found in the environment (and in humans) may induce various responses, but only to a low or moderate extent compared to the most potent PCBs.

In order to increase the resolution in the QSAR models for future use in risk assessments, the studied class of chemicals has to be divided into sub-groups. One feasible strategy is to use the data completed in the screening phase to select compounds for further studies of the chemical domain of the highest probable activity. Such studies have been initiated for the PCBs potency to induce CYP1A related activities and estrogenic effects, and could be used for mapping other chemical domains of the PCBs. Challenges in this approach include optimising the selection procedure to identify representative compounds from the pertinent sub-region of the chemical domain. D-optimal design may be an appropriate tool for identifying such sets of compounds. These models will by definition include compounds with less structural variation than that found in the full set of compounds, and thus descriptors are needed that reflect fine variation in physico-chemical characteristics. For modelling of very specific toxic mechanisms of action, such as interaction with steroid receptors, three-dimensional QSARs using comparative molecular field analysis (CoMFA) techniques are promising

(Tong *et al*. 1997; Waller *et al*. 1995). In addition, spectroscopic techniques such as mass and infrared spectroscopy and *ab initio* calculations may provide structure-specific electronic information. Further, the strategy applied in this project could be used for other environmental contaminants. These include groups of compounds such as the polybrominated diphenylethers, the toxaphenes and those compounds listed as potential endocrine disrupters.

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