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PCBs: Structure–Function Relationships and Mechanism of Action

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Numerous reports have illustrated the versatility of polychlorinated biphenyls (PCBs) and related halogenated aromatics as inducers of drug-metabolizing enzymes and the activity of individual compounds are remarkably dependent on structure. The most active PCB congeners, 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl, are substituted at both *para* and at two or more *meta* positions. The four coplanar PCBs resembled 3-methylcholanthrene (3-MC) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in their mode of induction of the hepatic drug-metabolizing enzymes. These compounds induced rat hepatic microsomal benzo(a)pyrene hydroxylase (aryl hydrocarbon hydroxylase, AHH) and cytochromes P-450a, P-450c and P-450d. 3,4,4',5-Tetrachlorobiphenyl, the least active coplanar PCB, also induced dimethylaminoantipyrine *N*-demethylase and cytochromes P-450b+e and resembled Aroclor 1254 as an inducer of the mixed-function oxidase system. Like Aroclor 1254, all the mono-*ortho*- and at least eight di-*ortho*-chloro analogs of the coplanar PCBs exhibited a "mixed-type" induction pattern and induced microsomal AHH, dimethylaminoantipyrine *NM*-demethylase and cytochromes P-450a–P-450e. Quantative structure–activity relationships (QSARs) within this series of PCBs were determined by comparing their AHH induction potencies (EC_{50}) in rat hepatoma H-4-II-E cells and their binding affinities (ED_{50}) for the 2,3,7,8-TCDD cytosolic receptor protein. The results showed that there was an excellent correlation between AHH induction potencies and receptor binding avidities of these compounds and the order of activity was coplanar PCBs (3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyls) > 3,4,4',5-tetrachlorobiphenyl ~ mono-*ortho* coplanar PCBs > di-*ortho* coplanar PCBs. It was also apparent that the relative toxicities of this group of PCBs paralleled their biological potencies.

The coplanar and mono-*ortho* coplanar PCBs also exhibit differential effects in the inbred C57BL/6J and DBA/2J mice. These compounds induce AHH and cause thymic atrophy in the former "responsive" mice whereas at comparable or higher doses none of these effects are observed in the nonresponsive DBD/2J mice. Since the responsiveness of these two mice strains is due to the presence of the *Ah* receptor protein in the C57BL/6J mice and its relatively low concentration in the DBA/2J mice, the results for the PCB cogeners support the proposed receptor-mediated mechanism of action.

Although the precise structural requirements for ligand binding to the receptor have not been delineated, the halogenated aromatic hydrocarbons which exhibit the highest binding affinities for the receptor protein are approximate isostereomers of 2,3,7,8-TCDD. 2,3,4,4',5-Pentachlorobiphenyl elicits effects which are qualitatively similar to that of TCDD and the presence of the lateral 4'-substituent is required for this activity. Thus the 4'-substituted 2,3,4,5-tetrachlorobiphenyls have been used as probes for determining the substituent characteristics which favor binding to the receptor protein. Multiple regression analysis of the competitive binding EC_{50} values for 13 substituents gave the following equation: $\log (1/EC_{50}) = 1.53\sigma + 1.47\pi + 1.09\ HB + 4.08$ where σ is electronegativity, π is hydrophobicity, HB is hydrogen bonding and r is the correlation coefficient ($r = 0.978$). The utility of this equation in describing ligand:receptor interactions and correlations with toxicity are being studied with other halogenated hydrocarbons and PAHs.

Introduction

Polychlorinated biphenyls (PCBs) are highly stable organic-soluble industrial compounds which have been widely used as dielectric and heat transfer fluids, plasticizers, wax extenders and flame retardants (*1*). PCB residues have been identified in almost every component of the global ecosystem including rivers and lakes, the atmosphere, fish wildlife, human adipose tissue, blood and breast milk (*2–10*). Commercial PCBs, in common with other halogenated aromatics such as the polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and polybrominated biphenyls (PBBs), elicit a number of common toxic and biologic effects (*11–15*). PCBs typically cause thymic atrophy, a wasting syndrome, immunotoxic responses, reproductive problems,

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porphyria and related liver damage. In PCB-exposed animals, these effects are preceded by the induction of numerous enzymes including the hapatic and extrahepatic drug-metabolizing enzymes.

Inducers of this latter enzyme system have traditionally been divided into two main classes, typified by phenobarbital (PB) and 3-methylcholanthrene (3-MC) (*16–18*). Pretreatment of rats with PB-type inducers enhances numerous hepatic drug-metabolizing enzyme activities including several cytochrome P-450-dependent monooxygenases (e.g., dimethylaminoantipyrine (DMAP), ethylmorphine and related *N*-dealkylases, biphenyl-4-hydroxylase, aldrin epoxidase and several *O*-dealkylases). In contrast, 3-MC and 3-MC-type inducers enhance hepatic microsomal benzo(a)pyrene hydroxylase, ethoxyresorufin *O*-deethylase (EROD) and several other cytochrome P-450-dependent monooxygenases. The differential effects of 3-MC, PB and other monooxygenase enzyme inducers are directly related to their induction of specific cytochrome P-450 isozymes (*19–23*). 3-MC and related compounds induce cytochromes P-450a, P-450c and P-450d, whereas PB and PB-type chemicals induce cytochromes P-450a, P-450b plus P-450e (*21*). Aroclor 1254 induces both the PB- and 3-MC inducible monooxygenases (*24–26*) and cytochromes P-450a–P-450e (*21,27–31*) and resemble PB plus 3-MC (coadministered) in its mode of drug-metabolizing enzyme induction. In retrospect, this bifunctional or "mixed-type" induction pattern for Aroclor 1254 was not unexpected since the commercial PCB formulations are complex mixtures of isomers and congeners which contribute to the observed induction activity (*1,32–34*).

The mechanism of action of the toxic halogenated aromatics has primarily been derived by investigating the effects of 2,3,7,8-TCDD and related PCDD isomers and congeners and several highly toxic halogenated aromatics that are approximate isostereomers of 2,3,7,8-TCDD (*11,12,35–38*). Several significant correlations were noted for the PCDD series of compounds, including: (1) the most active PCDDs are substituted at the 2,3,7, and 8 lateral positions (i.e., 2,3,7,8-TCDD), and Cl substitution or removal of the lateral Cl groups gives compounds with diminished activity; (2) there is an excellent correlation between the toxicity of several PCDDs, their AHH-inducing potency and their relative affinities for a high affinity, low capacity hepatic cytosolic receptor protein; (3) the differential effects of the PCDDs and related toxic halogenated aromatics on genetically inbred nonresponsive DBA/2J and responsive C57BL/6J mice suggest that receptor protein binding by these toxic ligands is the first critical step which initiates the complex sequence of events leading to the observed biologic and toxic responses. In C57BL/6J mice the soluble binding protein (i.e., the *Ah* receptor) also plays a role in mediating the activities of several aromatic hydrocarbons such as 3-MC and benzo(a)pyrene.

The most toxic PCB congeners, namely, 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl can assume coplanar conformations and are approximate isostereomers of 2,3,7,8-TCDD. These three PCBs are potent AHH inducers (*39–43*), elicit biologic and toxic effects comparable to those reported for 2,3,7,8-TCDD, and the results support a common mechanism of action for the toxic PCBs and PCDDs. Based on PCB structure–activity relationships (SARs), it was concluded that the most active compounds must be substituted at the *para* and at least one *meta* position of both phenyl rings and must not contain any *ortho*-chloro substituents (*40,41*). Unfortunately, the proposed PCB SARs define a subset of three compounds that are highly active but are present as only trace components in the commercial PCB mixtures (*44*). It is conceivable that the observed biologic and toxic effects of PCB formulations may either be due to the trace levels of the coplanar PCBs, and/or the presence of highly toxic PCDF impurities (*45,46*) or the presence of other toxic PCBs which have not been defined by the proposed structure–activity correlations (*40,41*). Studies in our laboratory with Aroclor 1254 showed that after Florisil column chromatography, the cleaned-up (PCDF-free) commercial PCBs and the crude mixture exhibited comparable AHH induction potencies. This suggested that unidentified toxic PCBs may be present in the commercial mixtures and a more comprehensive PCB structure–activity study was undertaken to resolve this problem.

Coplanar PCBs

A systematic study of the effects of structure on the AHH-inducing activity of PCBs would require the synthesis of all 209 isomers and congeners. However, structural considerations dictate that only PCBs which are substituted in the *para* and *meta* positions will exhibit maximum coplanar conformational character and approximate the relatively flat structure of 2,3,7,8-TCDD. Moreover, of the 20 possible coplanar PCBs, it is apparent from several studies that AHH-inducing activity is only observed for compounds substituted at both *para* positions (*40,41,47*). Figure 1 thus summarizes the structures of all the 4,4'-dichlorobiphenyl-substituted PCBs and represents a subset of the group of coplanar compounds that are most likely to exhibit activity. Several reports have shown that 4,4'-dichlorobiphenyl resembles PB in its mode of enzyme induction (*40,41,48,49*); the stepwise introduction of *meta*-chloro substituents into this base 4,4'-dichlorobiphenyl molecule has significant effects on the biologic and toxic effects of the resultant PCBs. 3,4,4'-Tri- and 3,4,4',5-tetrachlorobiphenyl exhibit mixed-type induction activity in male Long-Evans and Wistar rats, and this is characterized by the induction of hepatic microsomal AHH, DMAP *N*-demethylase and cytochromes P-450a– P-450e (*47,50,51*). In contrast the 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyls are more selective in their enzyme induction activity and induce microsomal AHH and cytochromes P-450a, P-450c and P-450d. A semiquantitative comparison of the activity

of the coplanar PCBs is apparent from their potencies as inducers of cytochromes P-450c plus P-450d; the order of increasing inducing activity was 3,4,4'-tri- < 3,4,4',5-tetra- < 3,3',4,4'-tetra- < 3,3',4,4',5-penta < 3,3',4,4',5,5'-hexachlorobiphenyl (Table 1). The quantitative SAR within this group of coplanar PCBs was determined by measuring their AHH/EROD induction potencies with rat hepatoma H-4-II-E cell in culture and their relative binding affinities for the male Wistar rats hepatic cytosolic receptor protein (*51,52*). The EC_{50} and values for enzyme induction were estimated from dose-response studies; the ED_{50} for binding affinities were derived from the effects of different concentrations of each PCB congener in displacing (competitively) bound [^{3}H]TCDD from the receptor protein. Figure 2 summarizes the results of these studies and illustrates the excellent correlation between AHH induction potencies and receptor binding affinities for this group of coplanar PCBs.

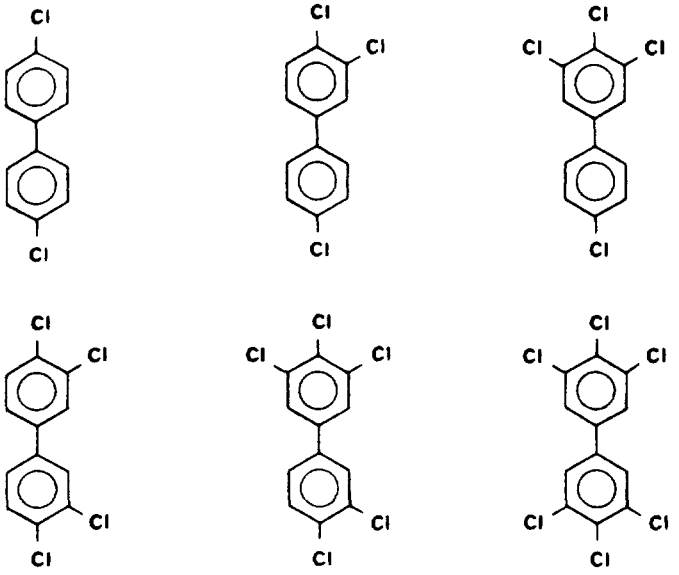

FIGURE 1. Structures of all the coplanar PCB congeners substituted at both *para* and 0-4 *meta* positions.

Several studies reported the toxic effects of the 4,4'-dichlorobiphenyl-substituted coplanar PCBs and these data confirm the correlation between the *in vitro* AHH/EROD induction and receptor binding activities and toxicity (*39,40,53,54*). 4,4'-Dichlorobiphenyl is relatively nontoxic and does not induce AHH; 3,4,4'-trichlorobiphenyl, the least active AHH inducer within this group of coplanar PCBs, does not cause thymic atrophy in male Long-Evans rats at a dose of 500 μmole/kg; in contrast, pretreatment with the same dose level of the 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3',4,4',5-penta and 3,3',4,4',5,5'-hexachlorobiphenyls resulted in significantly reduced thymus weights in the Long-Evans rats (*50*). Several additional papers clearly show that the more active 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyls elicit toxic responses typical of 2,3,7,8-TCDD including weight loss after exposure, hepatonecrosis, porphyria, thymic atrophy and reproductive toxicity (*38,41,50,53–55*).

Mono-*ortho* Coplanar PCBs

The introduction of a single *ortho*-chloro substituent into the biphenyl ring results in decreased coplanarity between the two phenyl rings due to steric interactions between the bulky *ortho*-chloro and hydrogen substituents. It was initally assumed (*40,41*) that the reduction in coplanarity for these compounds would prevent their binding to the cytosolic receptor and thus eliminate

Table 1. PCBs: Summary of structure–function relationships.

PCB structures (*n*)	Cytochromes P-450 induction, % of control[a]		Relative activity		
			AHH induction, %		Receptor binding, %
	P-450c + P-450d	P-450b + P-450e	*In vivo*[b]	*In vitro*[c]	
Coplanar PCBs—I[f] (3)	4100–1800	No induction	+ + +	100–1	100–35
Coplanar PCBs—II[g] (2)	1500–1100	1400–600	+ +	3×10^{-2}[h]	0.5[h]
Mono-*ortho* coplanars (8)	2400–750	4700–2600	+ +	$0.3 - 2.4 \times 10^{-5}$	6–1.5
Di-*ortho* coplanars (12)	900–250	6300–1000	+	Inactive	< 0.3[e]
2,2',4,4',5,5'-Hexachlorobiphenyl	No induction	7300	Inactive	Inactive	< 0.3
2,3,7,8-TCDD	3500	No induction	+ + + + +	400	2500

[a]Male Long-Evans rats (dose: 500 mole/kg).
[b]Male Wistar rats (dose: 300 mole/kg).
[c]Rat hepatoma H-4-II-E cells.
[d]Determined by the competitive displacement of [^{3}H] TCDD bound to male Wistar rat hepatic cytosol.
[e]Represents nonspecific binding.
[f]3,3',4,4'-Tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl.
[g]3,4,4'-Tri- and 3,4,4',5-tetrachlorobiphenyl.
[h]Determined only for 3,4,4',5-tetrachlorobiphenyl.

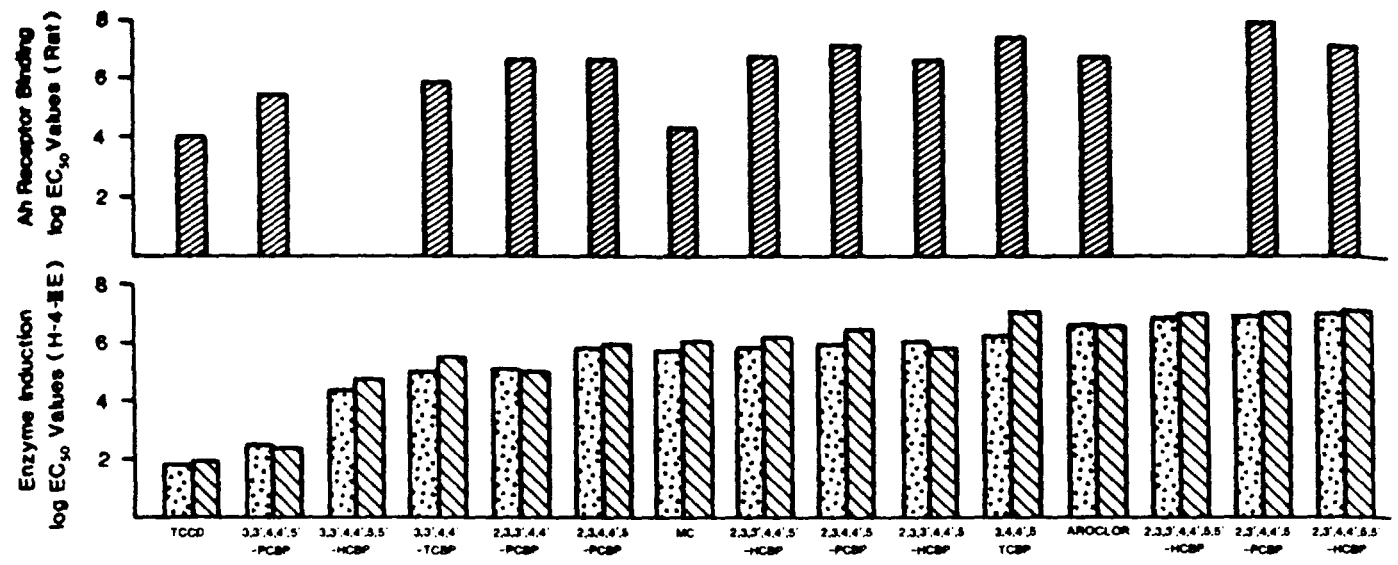

FIGURE 2. AHH/EROD induction potencies (bottom) and cytosolic receptor protein binding affinities of coplanar and mono-*ortho* coplanar PCB isomers and congeners. ☐ EROD; ☐ AHH; ☐ *Ah* receptor binding.

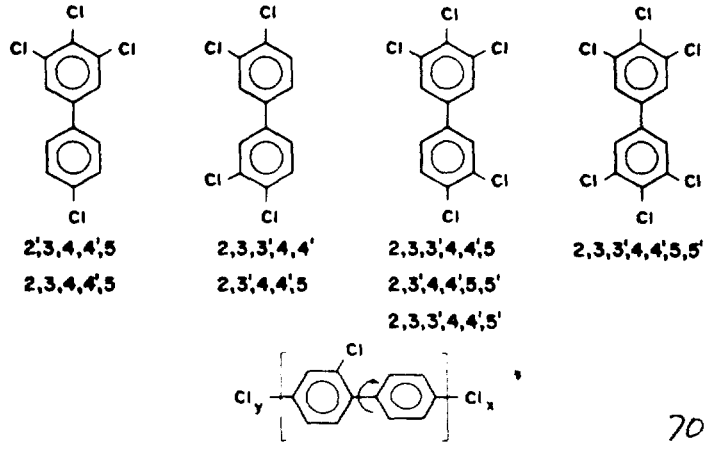

FIGURE 3. Structures of the most active coplanar PCB congeners (top) and the substitution patterns of their corresponding mono-*ortho* analogs (bottom).

their toxic and biologic effects. The effects of *ortho* substituents on PCB activity were tested by synthesizing all the mono-*ortho* analogs of the most active coplanar PCBs (i.e., 3,4,4,5,-tetra-, 3,3,4,4-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl) (Fig. 3) and determining the mixed-function oxidase enzyme-inducing activities in immature male Wistar and Long-Evans rats (*50,56,57*). All of these compounds induce AHH and DMAP *N*-demethylase in the Wistar rats and cytochromes P-450a–P-450e in the Long-Evans rats. It was apparent that the mono-*ortho* analogs of the coplanar PCBs resembled PB plus 3-MC (coadministered) and Aroclor 1254 in their mode of drug-metabolizing enzyme induction. Figure 2 illustrates the *in vitro* quantitative SAR within this group of PCBs for AHH/EROD induction potencies and receptor binding affinities. A comparison of the coplanar and mono-*ortho* coplanar PCBs clearly shows that the *ortho*-chloro substituent diminishes but does not eliminate binding affinities of the mono-*ortho* coplanar PCBs are accompanied by decreased AHH/EROD induction potencies. These results identify the structures of the active chlorinated biphenyls components in the commercial PCB. Several mono-*ortho* coplanar PCBs, including 2,3,3',4,4'-penta-, 2,3',4,4',5-penta-, 2,3,3',4,4',5-hexa- and 2,3,3',4,4',5,5'-heptachlorobiphenyl have been identified in several commercial formulations including the Aroclors, Phenoclors and Kanechlors (*1,32–34*).

The toxicities of the mono-*ortho* coplanar PCBs have not been systematically investigated; however, many of these compounds elicit toxic effects similar to 2,3,7,8-TCDD and related halogenated aromatics. For example: 2,3',4,',5-penta-, 2,3,3',4,4'-penta-, 2,3,3',4,4',5-hexa- and 2,3,3',4,4',5'-hexachlorobiphenyl cause thymic atrophy in rats (*50*). Administration of 2,3,3',4,4' -pentachlorobiphenyl to mice and rats results in a wasting syndrome (weight loss), edema, liver lipid accumulation, extensive hepatic damage and splenic atrophy (*58*); 2,3',4,4',5-pentachlorobiphenyl causes 100% embryo mortality in eggs from pullets receiving the PCB in their diet at a level of 20 ppm (*59*); administration of 2,2',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl to rats caused increased liver weights, increased liver lipids and thymic atrophy (*60*). **These data indicate that at least five of the mono-*ortho* analogs of the coplanar PCBs elicit toxic effects which resemble (qualitatively) 2,3,7,8-TCDD, and several of these compounds (2,3,3',4,4'-penta, 2,3',4,4',5-penta-, 2,3,3',4,4',5-hexachlorobiphenyl) have been identified in commercial PCBs and as residues in human tissues (*32,61,62*).** Future research should establish the quantitative contributions of this group of compounds to the toxic and biologic effect of commercial PCBs and the PCB residues which persist in human tissues.

Di-*ortho* Coplanar PCBs

The steric interactions of two-*ortho*-chloro substituents would markedly inhibit biphenyl ring coplanarity and presumably decrease binding to the cytosolic receptor protein. Thus the di-*ortho*-substituted PCBs should not be inducers of AHH (or cytochromes P-450c and P-450d) or elicit the toxic responses typical of 2,3,7,8-TCDD. However, these conclusions did not correlate with several conflicting reports in the literature which indicated that thre di-*ortho* coplanar PCBs, namely 2,2',3,3',4,4'-, 2,2',3,4,4',5,5'- and 2,2',4,4',5,5'-hexachloro biphenyl, either induced microsomal AHH or resembled PB in their mode of induction (*40,41,63–65*). The problem was partially resolved when 2,3,7,8-tetrachlorodibenzofuran, a contaminant formed in the synthesis of 2,2',4,4',5,5'-hexachlorobiphenyl, was shown to be the active AHH-inducing component of the commercially available isomeric hexachlorobiphenyl (*O65*). Since several di-*ortho*-substituted PCBs, including 2,2',4,4',5,5'- and 2,2',3,4,4',5'-hexachlorobiphenyl, are major components of the commercial mixtures (*32–34*), the microsomal mixed-function oxidase enzyme induction activities of all the di-*ortho* coplanar PCBs analogs were determined (Fig. 4). The results (*66,67*) indicated that in male Wistar rats (dose level: 300 μmole/kg) **at least five members of this group, namely 2,2',3,3',4,4'-hexa, 2,3,3',4,4',6-hexa-, 2,2',3,4,4',5'-hexa, 2,3,4,4',5,6-hexa- and 2,2',3,3',4,4',5-heptachlorobiphenyl**, exhibited a mixed-type induction pattern. Using the more sensitive cytochrome P-450 isozyme immunoquantitation assay, it was shown that these five PCBs and at least two additional compounds, 2,3',4,4',6-penta and 2,3',4,4',5',6-hexachlorobiphenyl, induced cytochromes P-450a–P-450e in male Long-Evans rats (dose level: 500 μmole/kg) (*50*). Since not all the di-*ortho* coplanar PCBs were evaluated as inducers of cytochrome P-450c, the isozyme associated with AHH induction, it is possible that other members of this group may also induce this hemoprotein. 2,2',4,4',5,5'-Hexachlorobiphenyl was the only di-*ortho* coplanar PCB that did not induce cytochromes P-450c and P-450d but, like PB, induced cytochromes P-450a and P-450b + P-450e (*50*).

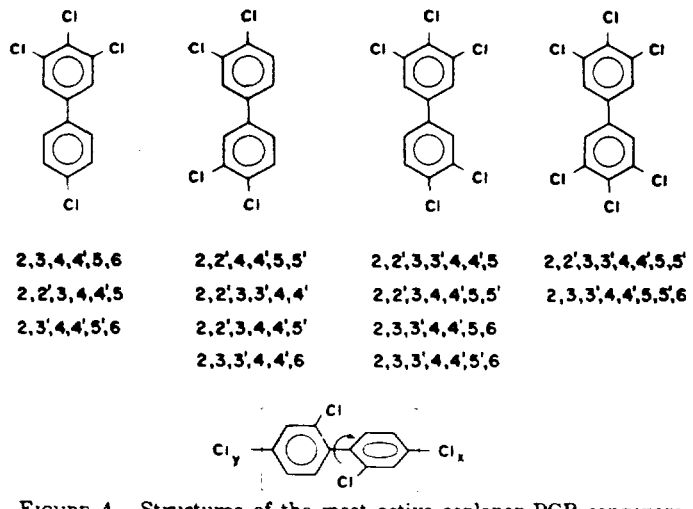

FIGURE 4. Structures of the most active coplanar PCB congeners (top) and the substitution patterns of their corresponding di-*ortho* analogs (bottom).

The di-*ortho* coplanar PCBs exhibited low binding affinities for the cytosolic receptor protein (*52*) and were relatively inactive as AHH/EROD inducers in rat hepatoma H-4-II-E cells (*51*). Thus, no meaningful quantitative SAR were derived for these compounds.

The toxicity of the di-*ortho* coplanar PCBs has not been systematically investigated; however, two members of this group, 2,2',3,3',4,4'- and 2,2',3,4,4',5'-hexachlorobiphenyl are porphyrinogenic in rats after long-term feeding studies (*64*). Both of these compounds are among the most active di-*ortho* coplanar PCB inducers of rat hepatic microsomal AHH and cytochromes P-450c. Based on the relatively low activities of this group of compounds, future **toxicologic research should focus on the effects of 2,2',3,4,4',5'-hexachlorobiphenyl** since this isomer is a major component of commercial PCBs and preferentially bioconcentrates in human blood, adipose tissue and breast milk (*32–34,61,62*).

Differential Effects on Genetically Inbred C57BL/6J and DBA/2J Mice

This paper has focused on the remarkable effects of structure on the biologic and toxic potencies of PCBs and the parallel between the effects of PCBs and the related toxic halogenated aromatics. It is evident that for both PCBs and PCDDs the most active compounds are approximae isostereomers of 2,3,7,8-TCDD and there is an excellent correlation between the cytosolic receptor binding affinities, AHH induction potencies and toxicities of these halogenated aromatics. Further evidence for a common mechanism of action of PCBs and PCDDs is suggested by the differential effects of these compounds on the responsive C57BL/6J and nonresponsive DBA/2J inbred mice (*38,40,42,68–70*). Administration of the coplanar PCBs or 2,3,7,8-TCDD to C57BL/6J mice results in the induction of hepatic microsomal AHH, immunotoxicity and weight loss whereas the nonresponsive mice are much less susceptible to the effects of these compounds (e.g., Fig. 5). 2,3,4,4',5-Pentachlorobiphenyl, a mono-*ortho* coplanar PCB induces AHH and causes thymic atrophy in the responsive C57BL/6J mice whereas no significant AHH induction or thymic atrophy is observed in the DBA/2J mice (*62*). Comparable results have been obtained for most of the mono-*ortho* coplanar PCBs (*70*) and Aroclor 1254 and this suggests that this group of compounds and their coplanar PCB precursors all act through a common mechanism. Support for the receptor-mediated mechanism of action of 2,3,7,8-TCDD has been derived from comparable studies with the two inbred mice strains (*36–*

38). The responsive C57BL/6J mice contain relatively high levels of the *Ah* receptor in hepatic and extrahepatic tissues whereas relatively low levels of this protein have been detected in the nonresponsive DBA/2J mice (71). Therefore the differential effects of coplanar and mono-*ortho* coplanar PCB congeners in the two strains of mice lend further support to the proposed receptor-mediated mechanisms of action for the toxic halogenated aromatics.

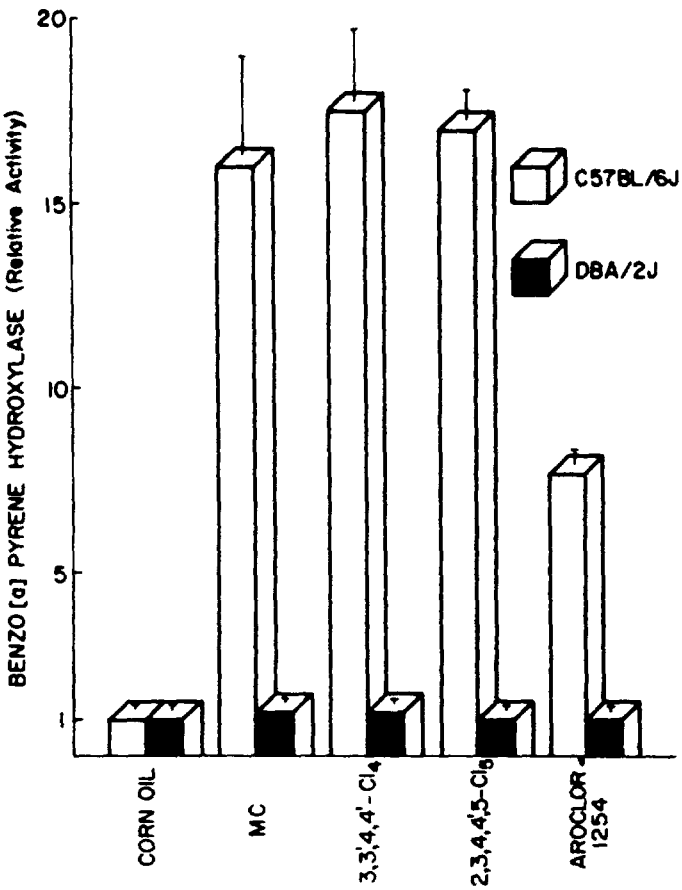

FIGURE 5. Benzo(a)pyrene hydroxylase induction in C57BL/6J and DBA/2J mice by corn oil, 3-MC, 3,3',4,4'-tetrachlorobiphenyl, 2,3,4,4',5-pentachlorobiphenyl and Aroclor 1254.

Receptor: Ligand Interactions: QSAR

The qualitative and quantitative SARs observed for PCB isomers and congeners also support the proposed mechanism of action for the toxic halogenated aromatics (Fig. 6). This model is based on the mechanism of action of steroid hormones in which the process is initiated by the noncovalent interaction between a ligand (i.e., a coplanar toxic halogromatic) and a receptor protein. The ligand: receptor binding complex is then translocated into the nucleus, and presumably interacts with a specific region of the nuclear DNA. These events trigger the *de novo* synthesis of m-RNA and protein which ultimately leads to the pleiotypic responses observed in the host animals. Although there is considerable evidence for a receptor-mediated mechanism of action for PCBs, the mechanistic details for the individual steps involved in this process are not well defined. This review has focused on two aspects of this model, namely, the receptor:ligand interaction and the induction of cytochrome P-450-dependent monooxygenases.

McKinney and co-workers (72,73) have suggested that an important factor which facilitates the ligand:receptor protein interaction is the molecular polarizability of the lateral chloro substituents. A more comprehensive understanding of ligand:receptor interactions can be obtained by determining quantitative SARs within a series of structurally related compounds (74–77). This approach is routinely used in planning the synthesis of new agricultural chemicals and drugs and requires an

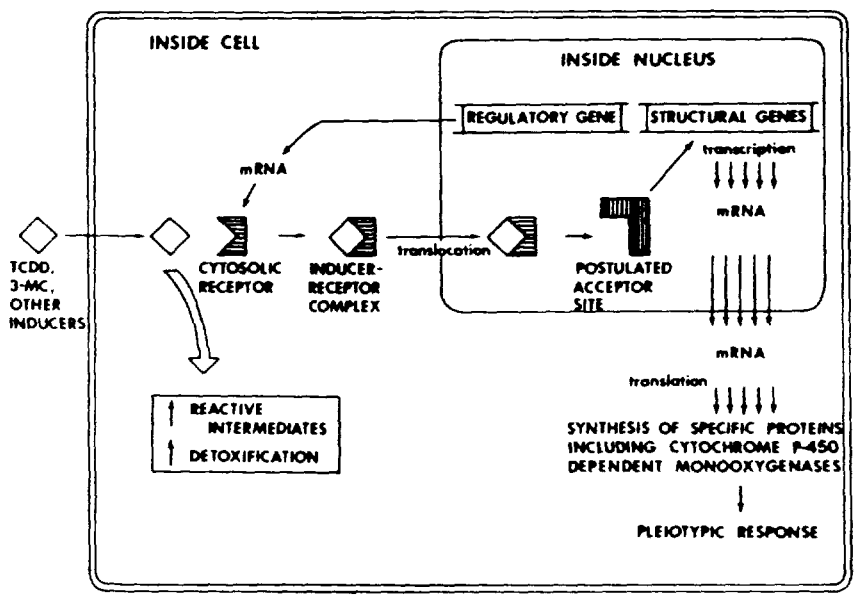

FIGURE 6. PCBs and related toxic halogenated aromatics: proposed mechanism of action.

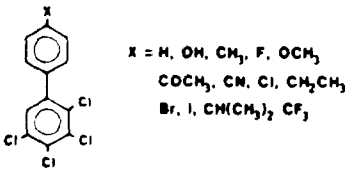

FIGURE 7. Structure of the model ligands.

active model chemical substrate that is amenable to structural manipulation at an active site. We have chosen 4'-substituted 2,3,4,5-tetrachlorobiphenyls (Fig. 7) as our first group of structurally related ligands for the following reasons (*78,79*): (1) 4'-substituted-2,3,4,5-tetrachlorobiphenyls contain a single variable substituent located at a critical lateral (*para*) position in the biphenyl nucleus, and the unsubstituted compound (Fig. 7, X = H) is relatively nontoxic and does not induce hepatic microsomal AHH; (2) like other toxic halogenated aromatics, the parent PCB congener, 2,3,4,4',5-pentachlorobiphenyl, induces microsomal AHH and cytochromes P-450c and P-450d, binds to the hepatic cytosolic receptor protein and causes thymic atrophy and weight loss in male Wistar rats; 2,3,4,4',5-pentachlorobiphenyl elicits similar effects in the responsive C57BL/6J mice but is inactive in the nonresponsive DBA/2J mice (Fig. 5); (3) this series of compounds can be synthesized by the Cadogan coupling of 4-substituted anilines and 1,2,3,4-tetrachlorobenzene to give a single biphenyl coupling product.

Figure 7 illustrates the structurally diverse 4'-substituted-2,3,4,5-tetrachlorobiphenyls which were used as ligands for the competitive receptor binding assays. The results obtained for 13 different halogenated biphenyls that differ only in the structure of the 4'-lateral substituent is summarized in Figure 8. Multiple linear regression analysis were performed with a FACOM M200 computer at the Data Processing Center of Kyoto University. Hydrophobic (π), electronic (δ) and hydrogen-binding (HB) accepting parameters were assigned for each substituent.

Hammett constants for the substituents were obtained from the literature (*75*) and values were calculated as described (*77*). The HB parameters for each substituent were assigned values of zero (for nonhydrogen bonders) or one (for hydrogen bonders) (*76*). Preliminary analysis of the receptor binding data indicated that three compounds with bulky 4'-substituents (X = phenyl, *n*-butyl and *t*-butyl) did not fit the equation. One apparent reason for their lack of fit is the van der Waals volume of the bulky substituents (> 35 cm/mole for these groups) which introduces a steric factor into the calculation. If the results for the bulky 4'-*n*-butyl-, 4'-*t*-butyl and 4'-phenyl-2,3,4,5-tetrachlorobiphenyls are omitted, thus multiple regression analysis of the data gave

$$\log (1/EC_{50}) = 139\,\sigma + 1.31\,\pi + 1.2\,HB + 4.20$$

for $n = 15$, SD = 0.31, $r = 0.916$, where r is the correlation coefficient and SD the standard deviation. This equation was recalculated by omitting the receptor binding time for the 4'-nitro- and 4'-*N*-acetylamino-2,3,4,5-tetrachlorobiphenyls. The latter compound did not fit the equation and there were problems in assigning an HB value for the nitro-substituted biphenyl due to possible in-plane and out-of-plane conformations. The recalculated equation for 13 compounds was

$$\log (1/EC_{50}) = 1.53\,\sigma + 1.47\,\pi + 1.09\,HB + 4.08$$

for $n = 13$, SD = 0.13, $r = 0.978$. The excellent correlation between binding affinities and substituent physical chemical parameters thus confirms the importance of steric, electronic, hydrophobic and hydrogen bonding factors in facilitating the interactions between toxic haloaromatics and the active binding state of the cytosolic receptor protein. The validity of the QSAR approach has been confirmed by determining the AHH/EROD induction potencies of the 4'-substituted-2,3,4,5-tetrachlorobiphenyls using rat hepatoma H-4-II-E cells in culture. With one exception, the order of induction potency for these compounds was identical to their order of binding affinities; the linear regression equation for this correlation is being calculated. 4'-Iso propyl-2,3,4,5-tetrachlorobiphenyl was much less active as an inducer than predicted from receptor binding data and this may be related to problems in cell uptake.

Preliminary results have shown that other substituted biphenyls, dibenzo-*p*-dioxins and dibenzofurans can also be used to probe *in vitro* QSARs and this data

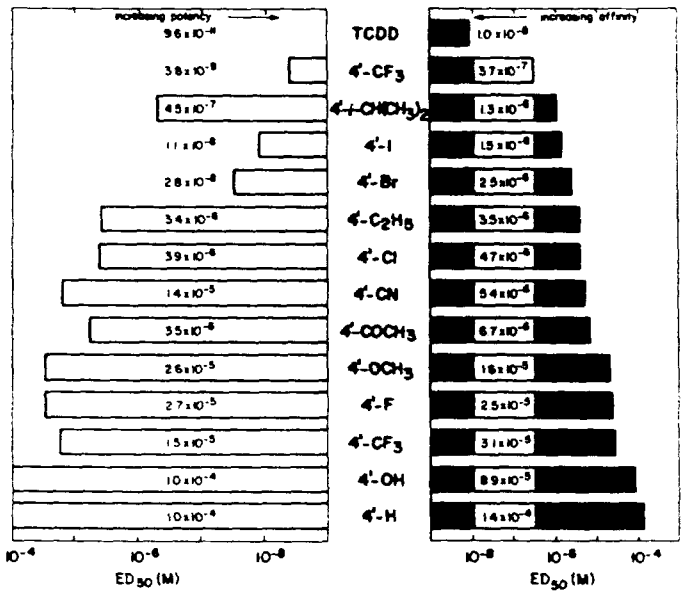

FIGURE 8. Comparative binding affinities (EC_{50}) of 4'-substituted-2,3,4,5-tetrachlorobiphenyls for the rat hepatic cytosolic 2,3,7,8-TCDD receptor protein (right); AHH induction potencies (EC_{50}) of 4'-substituted 2,3,4,5-tetrachlorobiphenyls in rat hepatoma H-4-II-E cells in culture (left).

will be used to further define the important interactions between halogenated aromatics and the 2,3,7,8-TCDD receptor protein. It is also apparent from *in vivo* studies that halogenated aromatics designed for *in vitro* QSAR studies can be used to investigate regulatory mechanisms for cytochrome P-450 isozymes and other drug-metabolizing enzymes. This research is now in progress.

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