POLYCHLORINATED BIPHENYLS (PCBs) AS INDUCERS OF THE HEPATIC MICROSOMAL DRUG-METABOLIZING ENZYMES – EFFECTS OF STRUCTURE ON ACTIVITY

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ABSTRACT

The effects of structure on the activity of polychlorinated biphenyls (PCBs) as inducers of hepatic drug-metabolizing enzymes was evaluated by comparing their modes of induction to the classical inducers, phenobarbitone (PB), 3-methylcholanthrene (MC) and PB plus MC (coadministered). Due to the structurally different PCB congeners which induce PB-type activity, it was not possible to accurately define structure-activity rules. It was noted that PCBs which possess 2,4-dichloro-,2,3,4-trichloro- and 2,4,5-trichloro-substitution patterns on both phenyl rings were the most potent PC-type inducers.

PCBs which induce MC-type (or aryl hydrocarbon hydroxylase, AHH) activity must be substituted at both *para* positions and at two or more *meta* positions (but not necessarily on the same phenyl ring). The compounds defined by these rules, namely, 3,3′, 4,4′-tetra-3,4,4′5-tetra-, 3,3′,4,4′,5-penta- and 3,3′,4,4′,5,5′,-hexachlorobiphenyl induce AHH activity in the rat and in rat hepatoma 4-H-II-E cells in culture and also bind to the cytosolic *Ah* receptor protein. Substitution of the four MC-type inducers with one *ortho*-chloro group yields a series of mixed-type inducers which also induce AHH activity and competitively displace 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from the *Ah* receptor protein. The di-*ortho* substituted analogs of 3,3′,4,4′,-tetra-, 3,4,4′,5-tetra, 3,3′,4,4′,5-penta- and 3,3′,4,4′,5,5′-hexachlorobiphenyl have also been tested in the rat and only five congeners, 2,3,4,4′,5-penta-, 2,2′,3,3′4,4′-hexa-, 2,2′,3,4,4′,5′,-hexa-, 2,3,4,4′,5,6-hexa- and 2,2′,3,3′,4,4′,5-heptachlorbiphenyl induced AHH activity at relatively high dose levels. The results are consistent with the proposed mechanism of enzyme induction by 2,3,7,8-TCDD and related halogenated aromatics.

I. INTRODUCTION

Polychlorinated biphenyls (PCBs) have been widely used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, plasticizers, flame retardants and dielectric fluids /1/. Commercial PCBs are prepared by the direct chlorination of biphenyl to give mixtures which are graded and sold according to their weight per cent chlorine. The diverse industrial applications of commercial PCBs are directly related to their physical and chemical properties which include (1) resistance to acids, bases, oxidation and reduction, (2) solubility in organic solvents, (3)

thermal stability, (4) excellent dielectric insulation properties and (5) non-flammability. Unfortunately these physico-chemical properties have contributed to the pollution problems associated with these chemicals. PCBs are remarkably resistant to photo- and bio-degradation and their lipophilic nature results in their bioconcentration and bio-accumulation. The first report of PCBs in human samples appeared in 1966 in which spurious peaks were observed in the analysis of environmental residues for DDT /2/. In the intervening years PCBs have been detected in almost every matrix tested including air, water, fish, wildlife, human adipose tissue, blood and breast milk /1-11/.

A major factor which must be considered in evaluating the toxic and biologic effects of PCBs is the complexity of the commercial PCB mixtures. There are 209 possible PCB isomers and congeners and some of the commercial products contain greater than 70 isomeric and congeneric components /4,12,13/. Moreover, due to the different physical and biological properties of individual PCBs, the composition of atmospheric and aquatic residues /11, 14/ and residues in wildlife and human tissues is markedly different from the parent commercial products /6-9, 11/. Thus an evaluation of the biologic and toxic impact of environmental PCBs requires analytical data on the composition of the mixtures and the activities of the individual PCB components.

II. PCB MIXTURES AS MICROSOMAL ENZYME INDUCERS

Many xenobiotics induce hepatic microsomal drug-metabolizing enzymes. Classically these inducers have been divided into two classes /15-17/; one group, typified by phenobarbitone (PB), induces a number of cytochrome P-450 dependent monoxygenases whereas the second class, typified by 3-methylcholanthrene (MC), induces the cytochrome P-448-dependent monooxygenases. Microsomal enzymes induced by PB or MC are readily distinguished from one another by their characteristic enzymic, spectral, antigenic and electrophoretic characteristics /15-20/. PB-induced microsomes exhibit increased dimethylaminoantipyrine (DMAP) N-demethylase (x2-3), benzo[a]pyrene hydroxylase (x2-3) and aldrin epoxidase (x3-5) activities whereas MC induction gives microsomal enzymes with enhanced benzo[a]pyrene, aryl hydrocarbon, hydroxylase (AHH) (x10-15), 4-chlorobiphenyl hydroxylase (x10-15) and ethoxyresorufin O-deethylase (x20-30) activities. PB and MC-induced microsomal hemoproteins are further differentiated by their different electrophoretic patterns and their reduced microsomal cytochrome

P-450 carbon monoxide and ethyl isocyanide binding difference spectra.

Commercial PCBs induce hepatic drug metabolizing enzymes and the more highly chlorinated Aroclors 1248, 1254 and 1260 are more potent than the lesser chlorinated products /21-24/. Several detailed studies of the mode of induction by Aroclor 1254 indicated that the properties of the induced microsomal enzymes were similar to those observed after simultaneous coadministration of PB plus MC. This mixed-type induction pattern has been confirmed in recent studies which show that Aroclor 1254 induces both cytochromes P-450b and P-450c which have also been identified as the major forms of cytochrome P-450 induced by PB and MC respectively /25/. Presumably the induction properties of the commercial PCBs are dependent on the activities of the individual PCB components and this has spurred numerous studies on the activity of PCB isomers and congeners as inducers of the hepatic drug metabolizing enzymes /26-33/.

III. PCBs as PB-TYPE INDUCERS: EFFECTS OF STRUCTURE ON ACTIVITY

It was clear from early reports that PCB isomers and congeners induce a broad range of hepatic microsomal drug metabolizing enzymes /21-38/. More detailed studies with a larger number of symmetrically-substituted PCBs suggested the following structural prerequisites for PCBs which exhibit PB-type activity /26, 27/:

1) The chlorinated biphenyls must be substituted in the *ortho* and *para* positions of both phenyl rings.

2) The addition of further *ortho* and *meta*-chloro substituents to the compounds defined in (1) does not apparently alter the qualitative aspects of the enzyme pattern. Thus PCB congeners which contain the 2,4-dichloro-2,3,4-trichloro- and 2,4,5-trichloro-substitution pattern on both phenyl rings exhibit a PB-type pattern of enzyme induction.

The structural requirements for PCBs as PB-type inducers were re-evaluated by systematically determining the requirements for *ortho*-, *meta*- and *para*- chloro substituents. The structural requirement for both *para* substituents was tested by evaluating the effects of a series of three hexachlorobiphenyl isomers, 2,3,3′,4,4′,5-hexachlorobiphenyl (HCBP-1), 2,3,3′,4′,5,6-hexachlorobiphenyl (HCBP-2) and 2,3,3′,5,5′,6-hexachlorobiphenyl (HCBP-3), as microsomal enzyme inducers (Figure 1). It was apparent from the results that pretreatment of rats with either HCBP-1, HCPB-2 (with one *para* substituent) or HCBP-3 (with no

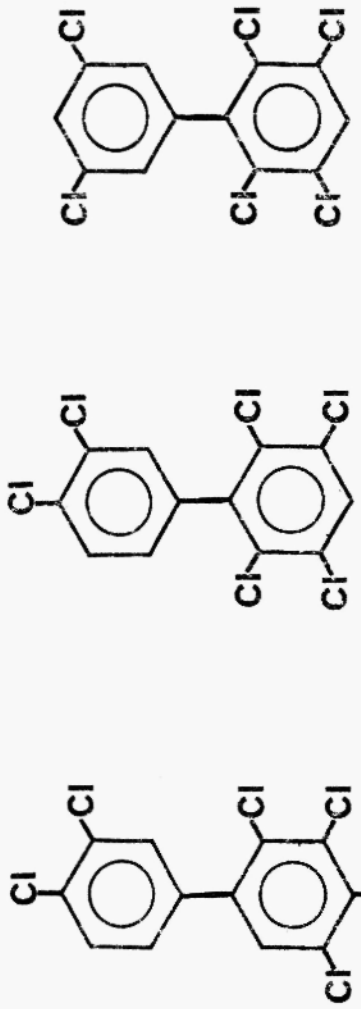

Fig. 1. PCB congeners which induce PB-type activity: effects of *para* substitution.

para substituents) resulted in a PB-type enhancement of hepatic microsomal B[a]P hydroxylase and DMAP N-demethylase activities and cytochrome P-450 content. A fourth isomer devoid of para substituents, 2,2′,3,5,5′,6-hexachlorobiphenyl exhibited only marginal PB-type activity. These data and previous results reported for 2,2′,3,3′,5,5′-hexachlorobiphenyl /37, 38/ demonstrate that *para* chloro substitution of biphenyl is not a structural requirement for PB-type induction.

It has also been reported that administration of 4,4′-dichlorobiphenyl to rats resulted in proliferation of hepatic smooth endoplasmic reticulum and increases in hepatic microsomal aniline hydroxylase and *p*-nitroanisole-O-demethylase activities /39/. These data suggested that PCB congener(s) without any *ortho* chloro substituents can be PB-type inducers. It is possible that the activity observed for 4,4′-dichlorobiphenyl may be associated with one or more of its metabolites. However, in the rat the major metabolites of 4,4′-dichlorobiphenyl are 4,4′-dichloro-3-biphenylol and 4′-chloro-4-biphenylol /40/, both of which lack *ortho* substituents. Furthermore, 3,3′-dichlorobiphenyl is also a weak PB-type inducer /36/. Due to such variability it is apparent that the precise structural requirements for PCBs as PB-type inducers of microsomal enzyme activities cannot be readily defined.

IV. PCBs as AHH INDUCERS: EFFECTS OF STRUCTURE ON ACTIVITY

A number of detailed studies on the effects of structure on the activity of polychlorinated dibenzo-*p*-dioxin (PCDD) isomers and congeners have shown that most potent AHH inducers, e.g. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), were the most toxic members of the PCDDs /41/. Moreover, a number of other halogenated aromatic compounds which are isosteric with TCDD, e.g. 3,3′,4,4′-tetrachlorazobenzene, 3,3′,4,4′,5,5′-hexachlorobiphenyl, 2,3,7,8-tetrachlorodibenzofuran and 3,3′,4,4′,5,5′-hexabromobiphenyl, were also active as AHH inducers and highly toxic /42/. The correlation between the toxicity of halogenated aromatic congeners and their potency as AHH inducers has prompted several studies on the effects of structure on the activity of PCBs as AHH inducers. Several reports by independent groups suggested the PCBs which induce MC-type activity must be substituted at both *para* positions and at least one *meta* position in each phenyl ring as exemplified by 3,3′,4,4′-tetrachlorobiphenyl (TCBP-1). Further *meta*-chloro substitution gives 3,3′,4,4′,5-pentachlorbiphenyl (PCBP-1)

or 3,3′,4,4′,5,5′-hexachlorobiphenyl (HCBP-4) which are also AHH inducers. The introduction of one or more *ortho*-chloro substituents into the nucleus of the three laterally-substituted PCB congeners abolished the induction of cytochrome P-448 dependent monooxygenases /26, 27, 36/. This was confirmed by the failure of 2,3,3′,4,4′,5,5′-heptachlorobiphenyl to induce microsomal AHH /27/. One apparent contradiction to these structure-activity rules was the reported activities of 2,2′,3,3′,4,4′- and 2,2′,4,4′,5,5′-hexachlorobiphenyl as MC-type inducers /32, 33/. A subsequent reinvestigation of the activity of 2,2′,4,4′,5,5′-hexachlorobiphenyl as a microsomal enzyme inducer has shown that the MC-type activity of a 99% pure commercial sample (prepared by the Ullmann coupling method) was due to contamination with the highly active 2,3,7,8-tetrachlorodibenzofuran /34/ (2378-TCDF). Other chlorinated dibenzofurans may also have contaminated the 2,2′,3,3′,4,4′-hexachlorobiphenyl sample.

The proposed structure-activity rules were, however, incompatible with the fact that although the higher chlorinated PCB mixtures were AHH inducers the concentrations of TCBP-1, PCBP-1 and HCBP-4 in these mixtures were undetectable (or unconfirmed) /4, 12, 13/. This suggested that the structure-activity rules were incomplete or that contaminants, such as 2,3,7,8-TCDF, which have been identified in some commercial PCBs were responsible for the activity of these mixtures as AHH inducers. Our reinvestigation of this problem has attempted to thoroughly evaluate the requirements for *ortho-*, *meta-* and *para*-chloro substituents on the activity of PCBs as AHH inducers /43-48/.

Requirements for meta-chloro substitution.

Previous reports indicated that PCB inducers of AHH must contain one or more *meta* substituents on each phenyl ring. The lack of activity of 3,4,4′-trichlorobiphenyl confirms the requirement for at least two *meta* substituents. However, the activity of 3,4,4′,5-tetrachlorobiphenyl (TCBP-2) as an AHH inducer indicates that the two *meta* substituents need not be located on different phenyl rings.

Requirements for ortho substitution.

The effects of *ortho* chloro substituents were determined by synthesizing and testing all the possible *mono-ortho*-chloro substituted analogs of TCBP-1, PCBP-1, HCBP-4 and TCBP-2. With one exception all of the resultant PCB congeners (Figure 2) were mixed-type inducers in the im-

Fig. 2. PCB congeners as AHH inducers: structures of PCBs derived from TCBP-1, PCBP-1, HCBP-4 and TCBP-2 containing one and two *ortho*-chloro substituents

mature male Wistar rat. Presumably the presence of one *ortho* substituent does not completely inhibit the coplanarity of the PCB phenyl rings which would yield rotamers or conformers isosteric with 2,3,7,8-TCDD. Confirmation of these results has been obtained using the highly inducible rat hepatoma H-4-II-E cell line; TCBP-1, PCBP-2, HCBP-4, TCBP-2 and their *ortho*-chloro substituted analogs induced cellular benzo[a]pyrene hydroxylase and ethoxyresorufin O-deethylase and their relative potencies in the rat and cell culture systems /49/ were comparable (Table 1). A further confirmation of the activity of these

TABLE 1. PCBs: Correlation between AHH induction in rats and rat hepatoma cells and binding to the Cytosolic *Ah* receptor protein

PCB Congener[a]	Relative Activities		
	Cell Culture[c]	In Vivo[d]	Receptor Binding[e]
3,3′,4,4′,5-PCBP	1000	xxxx	1000
3,3′,4,4′-TCBP	6.9	xxx	350
3,3′,4,4′,5,5′-HCBP	4.0	xxx	insol[b]
2,3,3′,4,4′-PCBP	2.7	xx	58
2′,3,4,4′,5-PCBP	2.4	xx	18
2,3,3′,4,4′,5-HCBP	0.11	xx	35
2,3,4,4′,5-PCBP	0.061	xx	53
2,3,3′,4,4′,5′-HCBP	0.034	xx	50
3,4,4′,5-TCBP	0.022	x	9
2,3,3′,4,4′,5,5′-HpCBP	0.0022	x	insol
2,3′,4,4′,5-PCBP	0.0022	x	28
2,3′,4,4′,5,5′-HCBP	0.0018	x	19

[a]TCBP-tetrachlorobiphenyl; PCBP-pentachlorobiphenyl; HCBP-hexachlorobiphenyl; HpCBP-heptachlorobiphenyl.

[b]Active but EC_{50} not obtained due to insolubility at higher concentrations.

[c]Rat hepatoma H-4-II-E cells in culture.

[d]Immature male Wistar rats.

[e]hepatic cytosol from immature male Wistar rats.

PCBs was investigated by comparing their relative binding affinities for the cytosolic *Ah* receptor protein /41/. This protein binds 2,3,7,8-TCDD and is believed to initiate the enzyme induction process associ-

ated with the halogenated aromatic ligand /50/. All the PCBs noted above competitively displace [^{3}H]-2,3,7,8-TCDD from the cytosolic receptor protein and in most cases their potencies as AHH inducers and as competitors for the receptor protein were comparable /51/. Although 2,3′,4,4′,5,5′-hexachlorobiphenyl appears to be a relatively weak inducer of cytochrome P-448 dependent monooxygenase enzyme activity there is also evidence that this compound may also resemble isosafrole in its mode of induction /52/. Thus the activity of 2,3′,4,4′,5,5′-hexachlorobiphenyl is being further investigated.

The di-*ortho*-chloro substituted PCB analogs of TCBP-1, PCBP-1, HCBP-4 and TCBP-2 (Figure 2) have also been synthesized and tested as AHH inducers in the immature male Wistar rat /47, 48/.

Two *ortho*-chloro substituents might be expected to have a more pronounced effect on the population of the planar conformers and, hence, on their activity as MC-type inducers. In an attempt to resolve conflicting reports in the literature, the effects of 2,2′,3,3′,4,4′-hexa-, 2,2′,4,4′,5,5′-hexa- and 2,2′,3′,4,4′,5-hexachlorobiphenyl on the hepatic microsomal drug-metabolizing enzymes were evaluated in the immature male rat. By comparison with the effects of the classical enzyme inducers, PB and MC, 2,2′,4,4′,5,5′-hexachlorobiphenyl was classified as a pure PB-type inducer. In contrast, 2,2′,3,3′,4,4′-hexachlorobiphenyl, irrespective of its synthetic route, exhibited PB-type and weak MC-type characteristics; the most prominent feature of the latter was a 7-fold increase in 4-chlorobiphenyl hydroxylase activity an MC-inducible enzyme. 2,2′,3′,4,4′,5-Hexachlorobiphenyl also resembled a mixed (PB + MC)-type inducer although its MC-type characteristics were more pronounced than those of 2,2′,3,3′,4,4′-hexachlorobiphenyl. Since these three hexachlorobiphenyls are disubstituted derivatives of the pure MC-type inducers, 3,3′,4,4′-tetrachlorobiphenyl, it is clear that the presence of two *ortho*-chlorines does not necessarily abolish MC-type character. The addition of two *ortho*-chlorines to the four MC-type PCB inducers (e.g. Figure 2) gave four mixed-type inducers, all containing a 2,3,4-trichloro substitution pattern (2,2′,3,3′,4,4′-, 2,2′,3,4,4′,5′-, 2,3,3′,4,4′,6- and 2,2′,3,3′,4,4′,5-hexachlorobiphenyl). In addition, the effects of 2,3,4,4′,5,6-hexachlorobiphenyl were also consistent with a mixed-type induction pattern. It is also conceivable that administration of higher dose levels (e.g. > 300 μmol $\cdot$ kg^{-1}) of these PCBs may indicate that other PCB congeners may be AHH inducers.

All PCBs which were AHH inducers contained two *para* substituents.

We conclude that PCBs which induce MC-type activity must be sub-

stituted in both *para* positions, at least two *meta* positions (but not necessarily on different phenyl rings) and can also contain one or two *ortho* chlorines, particularly if one phenyl ring contains a 2,3,4-trichloro substitution pattern.

Thus our experiments with PCB isomers and congeners confirm the correlation previously noted within the PCDD series, between their avidity in binding to the cytosolic receptor protein and their relative potencies as AHH inducers. There is evidence to suggest that, like the MC-type PCB inducers, some of the mixed-type inducers are more toxic than the PB-type inducers. For example, administration of the AHH inducers, 2,3,3′,4,4′-pentachlorobiphenyl (60 $mg \cdot kg^{-1}$), caused weight loss and death to rats, however the 2,2′,4,4′,5,5′-substituted PCB (150 $mg.kg^{-1}$) was inactive /53/.

Ax and Hansen /54/ observed 100% embryo mortality in eggs from chickens fed diets containing 20 $mg \cdot kg^{-1}$ (20 ppm) of the mixed-type inducer, 2,3′,4,4′,5-pentachlorobiphenyl, but only 10% mortality (i.e. control values) for the PB-type inducer, 2,2′,3,3′,5,5′-hexachlorobiphenyl. From long-term feeding experiments, Stonard and Greig /33/ determined that, in contrast to the PB-type inducer 2,2′,4,4′,5,5′-hexachlorobiphenyl, the mixed-type inducers, 2,2′,3,3′,4,4′- and 2,2′,3,4,4′,5′-hexachlorobiphenyl were porphyrinogenic to female rats. The mixed-type inducers, 2,3,3′,4,4′- and 2,3′,4,4′,5-penta- and 2,3,3′,4,4′,5-hexachlorobiphenyl, all cause fatty liver and thymic atrophy in immature male Wistar rats and it was suggested that the toxicity of these and other PCBs correlates with their ability to induce cytochrome P-448 /30/. It is possible, therefore, that the mixed-type inducers identified in the present study are major contributors to both the biologic and toxicologic properties of the commercial PCB mixtures and breast milk PCBs.

V. BREAST MILK PCBs as AHH INDUCERS

Table 2 summarizes all the PCBs expected to exhibit MC-type activity based on the above guidelines. A number of these isomers and congeners have been identified in commercial PCBs and in humans (blood, adipose tissue and breast milk). As noted in Table 2, there is a relatively high concentration of MC-type inducers which preferentially bioconcentrate in human breast milk. A recent study in our laboratory /46/ has shown that the dose effecting half-maximal (ED_{50}) induction of AHH activity for a reconstituted breast milk PCB sample was 12 μmol·

TABLE 2. Identification of PCB isomers and congeners in human breast milk /9, 46/

PCB Structure	PCB Concentration in Japanese Breast Milk (%)	PCB Concentration in Reconstituted Breast Milk Mixture %
2,4,4′-TCBP	8.4 ± 2.2	9.2
2,2′,5,5′-TCBP	2.0 ± 1.3	2.2
2,4,4′,5-TCBP	19.1 ± 2.6	20.1
2,2′,4,5,5′-PCBP	2.8 ± 0.9	3.0
2,3′,4,4′,5-PCBP*	11.8 ± 1.2	12.8
2,2′,3,4′,5,5′-HCBP	2.3 ± 0.3	–
2,3,3′,4,4′-PCBP*	3.5 ± 0.5	3.9
2,2′,4,4′,5,5′-HCBP	15.5 ± 0.4	16.3
2,2′,3,4,4′,5′-HCBP*	15.8 ± 0.7	16.9
2,2′,3,4′,5,5′,6-HCBP	3.2 ± 0.6	3.3
2,2′,3,4,4′,5′,6-HCBP	1.6 ± 0.3	1.7
2,3,3′,4,4′,5-HCBP*	2.1 ± 0.7	2.3
2,2′,3,4,4′,5,5′-HCBP	5.1 ± 0.7	5.7
2,2′,3,3′,4,4′,5-HCBP	2.3 ± 0.3	2.3
Total	95.5	99.9

*mixed-type inducers; the remainder are PB-type inducers.

kg^{-1} whereas the ED_{50} for Kanechlor 500, a commercial PCB mixture, was 87 $\mu mol \cdot kg^{-1}$. Thus, the increased biological potency of breast milk PCBs reflects the preferential bioconcentration of 2,3′,4,4′,5-penta-, 2,3,3′,4,4′-penta-, 2,3,3′,4,4′,5-hexa- and 2,2′,3′,4,4′,5-hexachlorobiphenyl which are mixed-type inducers. The toxicity and potential long term effects of reconstituted breast milk PCBs is currently being investigated in our laboratory.

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