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PCBs (Polychlorinated Biphenyls) Structure-Activity Relationships

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PCBs: STRUCTURE-ACTIVITY RELATIONSHIPS

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16. ABSTRACT

This report summarizes research on the chemical and toxicological characterization of PCB's. Results on the synthesis and characterizatin of all 209 PCB's and subsequent identification of individual PCB components in commercial mixtures and environmental samples are reported. This was essential for research relating the toxicity and biologic effects of commercial mixtures to chemical structure. The results of structure-activity research with the various congeners on several biological systems are also reported.

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are readily synthesized by the direct chlorination of biphenyl using iron salts and/or iodine as the catalyst. The degree of chlorination of the resulting mixture is controlled by the relative amount of chlorine used in the reaction and most commercial products are graded and sold by their weight per cent chlorine content [1]. PCBs have been widely used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, flame retardants, organic diluents and dielectric fluids. The unusual industrial versatility of PCBs is directly related to their physical properties which include: a) resistance to acids and bases, b) compatibility with organic materials, c) resistance to oxidation and reduction, d) excellent electrical insulating properties, e) thermal stability and f) non-flammability. Unfortunately, these physical properties coupled with their widespread use, relatively low acute toxicity and improper disposal has resulted in the contamination by PCBs of every component of the global ecosystem [1-5]. Moreover, the lipophilic nature and persistence of PCBs also contributes to their high bioaccumulation potential and their biomagnification in higher trophic levels of the food chain [6]. PCBs residues are routinely detected in fish, wildlife and human adipose tissue, blood and breast milk.

COMMON ANALYTICAL AND TOXICOLOGICAL PROBLEMS

The analysis of PCB residues in environmental samples present numerous problems which include the following:

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1. PCB formulations and environmental isolates are highly complex mixtures of isomers and congeners and
2. the composition of PCBs obtained from diverse environmental matrices are highly variable due to the different rates of biodegradation, vaporization, photodegradation and chemical degradation of the individual PCB isomers and congeners [6-8].

There are 209 possible PCB isomers and congeners and most of the commercial mixtures and environmental samples exhibit a multitude of peaks and this is particularly evident in the capillary gas chromatogram of the commercial PCB, Aroclor 1260. It has been common practice to quantitate environmental PCBs by comparing their packed column gas chromatograms with the patterns exhibited by known amounts of individual commercial PCBs or mixtures of these formulations [9,10]. This method relies on pattern matching using specific peaks for quantitation. It is clear that if the gas chromatograms of the environmental PCB residues cannot be "matched" with an appropriate cocktail containing known amounts of the commercial formulations then quantitation is not possible. Comparable analytical problems have been encountered in the analysis of other complex halogenated aromatic pollutants, and polynuclear aromatic hydrocarbons [11]. These problems experienced in PCB analysis by gas chromatography (GC) can be resolved in the following way:

1) the samples must be analyzed by high resolution capillary gas chromatography to ensure the maximum separation of the isomers and congeners,
2) all 209 PCB isomers and congeners must be available as analytical standards for the identification and quantitation of the individual components in the analyte.

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In collaboration with Dr. M. Mullin and several coworkers, considerable progress has been made on the synthesis and characterization of all 209 PCBs (Table 1) and this has resulted in the positive identification of the individual PCB components present in commercial mixtures and in environmental samples. Based on the numbering scheme proposed by Ballschmitter and Zell [13], Figure 1 illustrates the identification of the individual PCBs in Aroclor 1260 using the synthetic PCB standards [12].

Table 1. Progress in the Synthesis of PCB Isomers and Congeners

Cl Groups	1	2	3	4	5	6	7	8	9	10	Total
No. of Isomers	3	12	24	42	46	42	24	12	3	1	209
No. Synthesized or Available	3	9	18	40	42	40	24	12	3	1	192

Most reviews discussing the biologic and toxic effects of commercial PCBs note that the relative potencies of these formulations depends on their degree of chlorination and also on the species being used in the study [14,15]. The results clearly suggest that since the toxicities of the mixtures are variable, then the activities of the individual components of these mixtures may also be different. Research on the comparative biologic and toxic effects of PCBs has clearly illustrated the marked differences in the comparative toxicities of PCB congeners as noted below.

1) 2,3',4,4',5-Pentachlorobiphenyl is highly embryotoxic to chickens whereas 2,2',5,5'-tetra and 2,2',4,4',5,5'-hexachlorobiphenyl are relatively non-toxic [16].

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2) 3,3',4,4'-Tetrachlorobiphenyl is highly toxic to Rhesus macaques whereas the 2,2',5,5'-isomer elicits no clinical effects [17].

3) The toxic lesions caused by 3,3',4,4',5,5'-hexachlorobiphenyl in male mice were much more severe than those observed after administration of the 2,2',4,4',6,6'-, 2,2',4,4',5,5'- and 2,2',3,3',6,6'-hexachlorobiphenyls [18].

4) A comparison of the toxicity of 2,3,3',4,4'-penta- and 2,3',4,4'-tetrachlorobiphenyl showed the former congener to be highly active and the latter compound to be inactive [19].

5) Chronic administration of 2,2',3,3',4,4'- and 2,2',3,4,4',5'-hexachlorobiphenyl caused an accumulation of liver porphyrins whereas administration of the 2,2',4,4',5,5'-hexachlorobiphenyl had no effect on hepatic porphyrin levels [20].

6) 3,3',4,4',5- and 2,3',4,4',5-Pentachlorobiphenyl and 2,3,3',4,4',5-hexachlorobiphenyl cause thymic atrophy in rats whereas the 2,2',3,3',4,4',5- and 2,2',3,4,4',5,5'-heptachlorobiphenyls were not toxic [21].

It is evident that subtle changes in the structure of PCBs can result in marked differences in toxicities. Thus, an assessment of the potential long term effects of PCBs on biological systems will depend on the concentration of the individual toxic congeners which are present and which persist in the animal species. This necessitates not only a high resolution quantitative and qualitative analysis of PCB residues, but prior knowledge concerning the potential or actual toxicities of the individual PCBs.

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PCBs AS INDUCERS OF HEPATIC MICROSOMAL DRUG-METABOLIZING ENZYMES

One of the most characteristic biologic effects of the commercial PCBs is their induction of numerous hepatic microsomal drug-metabolizing enzymes in rodents and particularly the cytochrome P-450-dependent monooxygenases [14,22-25]. Administration of these mixtures results in increases in microsomal aniline hydroxylase, benzo[a]pyrene hydroxylase, biphenyl hydroxylases, halobiphenyl hydroxylases as well as numerous N- and O-dealkylases. The potent induction of these cytochrome P-450 dependent monooxygenases by Aroclor 1254 has resulted in the widespread use of PCB-induced microsomes as the source of metabolic activation for chemicals in the Ames test for their bacterial mutagenicity [26].

Inducers of microsomal monooxygenases are classically subdivided into two main categories [27-29]. One class is typified by phenobarbitone (PB) and the other by 3-methylcholanthrene (MC). PB-type inducers enhance microsomal N-demethylase (2-3-fold), NADPH cytochrome P-450 reductase (2-3-fold) biphenyl-4-hydroxylase (2-4-fold) and aldrin epoxidase (3-5-fold) whereas MC-type inducers enhance microsomal benzo[a]pyrene hydroxylase (8-12-fold), 4-chlorobiphenyl hydroxylase (8-10-fold), ethoxyresorufin O-deethylase (up to 30-fold) and biphenyl 2-hydroxylase (8-12-fold). The preferential induction of specific monooxygenases by MC- and PB-type inducers coupled with the different electrophoretic and spectral properties of their induced microsomal enzyme preparations clearly differentiates between the two classes of inducers. The enzyme induction properties of Aroclor 1254 and other commercial PCBs do not resemble that of either PB or MC but are similar to the pattern produced after co-administration of PB + MC [22-24]. More recent studies have shown that Aroclor 1254 induces cytochromes P-450b and P-450c which are also the major isozymes induced by PB and MC respectively [25]. The mixed-type

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induction activity of Aroclor 1254 was presumed to be due to the individual PB- and MC-type inducers present in this mixture.

PCBs AS MICROSOMAL MONOOXYGENASE ENZYME INDUCERS: EFFECTS OF STRUCTURE ON ACTIVITY

PB-Type Inducers

Initial studies suggested that PCBs which are PB-type inducers must contain at least two para and two meta substituents; any further substitution changes only the quantitative nature of the induction activity [30,31]. There is ample evidence for these correlation and some of the most active PB-type inducers are 2,2',4,4'-tetra-, 2,2',4,4',5,5'-hexa- and 2,2',3,3',4,4'-hexachlorobiphenyl. However it has subsequently been shown that PCB congeners which contain one or no para substituents, are also PB-type inducers [32]. Some of these compounds include 2,2',3,3',5,5'-hexachlorobiphenyl, 3,3'-dichlorobiphenyl, 2,3,3',5,5',6-hexachlorobiphenyl and 2,3,3',4',5,6-hexachlorobiphenyl. The requirement for at least two ortho chloro substituents conflicts with the reported activity of 4,4'-dichlorobiphenyl and 2,3',4,4'-tetrachlorobiphenyl as PB-type inducers [19,32]. Table 2 illustrates the structurally diverse group of PCBs which are classified as PB-type inducers and it is evident that there are no apparent structure-activity rules for PCBs which enhance this pattern of enzyme activity.

MC-Type Inducers

In 1977, two groups [30,31] published papers outlining structure-activity rules for PCBs as inducers of cytochrome P-448 dependent mono-

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Table II. PCBs as PB-Type Inducers

Congener	Number of Chloro Substituent			Reference
	Ortho	Meta	Para	
4,4'-dichlorobiphenyl	0	0	0	30, 31
2,3',4,4'-tetrachlorobiphenyl	1	1	2	19
2,2',4,4'-tetrachlorobiphenyl	2	0	2	30, 31, 36
3,3'-dichlorobiphenyl	0	2	0	31
2,3,3',4,5,5'-hexachlorobiphenyl	1	4	1	36

oxygenases (or aryl hydrocarbon hydroxylase, AHH). Their data indicated that PCBs which exhibit this type of induction activity must be substituted in both para positions and at least one meta position on both phenyl rings and not contain any ortho-chloro substituents. The three compounds defined by these rules, namely 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl are all potent AHH inducers and are approximate isostereomers of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic halogenated aromatic chemical and most potent inducer of microsomal AHH [33].

It was apparent that the proposed structure-activity rules did not explain the mixed-type induction activity of the commercial mixtures since the three AHH inducers are only trace components of the active commercial PCBs [3,13,33]. For this reason, the effects of structure on the activity of PCBs as AHH inducers were reevaluated using a series of highly purified synthetic PCB congeners [32,34-39].

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Requirements for Para Substituents

The requirements for para substituents were tested using a series of PCB congeners with one, two and zero para substituents and in all cases PCBs which were AHH inducers all contained two para-chloro groups. (Figure 2).

Requirements for Meta Substituents

The requirements for meta substituents were evaluated by testing all the possible meta-substituted PCBs derived from 4,4'-dichlorobiphenyl, a PCB which contains the required two para-chloro groups. Figure 2 denotes the five possible compounds in this series and only the 3,3',4,4'-tetra-, 3,4,4',5-tetra, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyls were AHH inducers. Thus one new PCB congener, namely 3,4,4',5-tetrachlorobiphenyl is added to the original list of three AHH inducers and it is apparent that two meta substituents are required for PCBs which induce AHH, however they can be positioned on both or the same phenyl ring.

Requirements for Ortho Substituents

A logical approach for evaluating the effects of ortho-chloro substituents on the activity of PCBs as AHH inducers is to synthesize and test all the possible mono- and diortho-substituted analogs of the four MC-type inducers. A summary of these compounds is given in Figure 3. All the mono-ortho substituted analogs were mixed-type inducers in the immature male Wistar rat and were inducers of AHH in rat hepatoma H-4-II-E cells in culture. Moreover many of these compounds, 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'-

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heptachlorobiphenyl have been identified as components of the commercial PCBs [3,13,33].

The effects of 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. Previous reports in the literature suggested that PCBs substituted in at least one meta and para position on each phenyl ring and at two ortho positions were strictly PB-type inducers as evidenced by the activity of the 2,2',3,3',4,4'- and 2,2',4,4',5,5'-hexachlorobiphenyl isomers [30,31]. Contradictory reports have suggested that these isomers also possess MC-type activity [20,40]. 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 (TCDF) [41].

In 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 [37]. 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 being a 7-fold increase in 4-chlorobiphenyl hydroxylase activity. 2,2',3',4,4',5-Hexachlorobiphenyl also resembled a mixed (PB + MC)-type inducer although its MC-type characteristics were more pronounced that those of 2,2',3,3',4,4'-hexachlorobiphenyl. Since these three hexachlorobiphenyls are di-ortho substituted derivatives of the pure

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MC-type inducer, 3,3',4,4'-tetrachlorobiphenyl, it is clear that the presence of two ortho chlorines does not necessarily abolish MC-type character. A detailed study of all the di-ortho substituted analogs of the four AHH inducers indicated that, in addition to the two PCB isomers noted above, 2,3,4,4',5,6-hexa-, 2,3,3',4,4',6-hexa- and 2,2',3,3',4,4',5-heptachlorobiphenyl were also mixed-type inducers [38].

Therefore the PCBs which induce MC-type activity must be substituted at both para positions, at least two meta positions (but not necessarily on different phenyl rings) and can also contain one ortho substituent; the addition of a second ortho chloro substituent to the nucleus of an MC-type inducer does not necessarily eliminate the qualitative aspects of this activity if one of the ortho substituents is part of a 2,3,4-trichlorophenyl substitution pattern.

PCBs Which Do Not Induce Microsomal Monooxygenases

It has also been shown that several PCBs containing one to three chlorine atoms or biphenyls chlorinated on only one of the phenyl rings are weak or inactive as inducer of cytochrome P-450 dependent monooxygenases [30,31]. Their lack of activity may be due to their more rapid rate of metabolism and clearance. Some of the more highly chlorinated octa-decachlorobiphenyls are also inactive [30,31,34]. Another group of PCBs which are inactive as AHH inducers contain 3,4 or 3,4,5 substitution on one phenyl ring with a 2,4,6- or 2,3,5-trichloro substitution pattern on the second ring [36,42,43]. These congeners do accumulate in the liver and contain one or two ortho-chloro groups, however, for some reason they are inactive as a microsomal monooxygenase inducer and are relatively non-toxic. The rationale for these results is not apparent.

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PCBs: CORRELATION BETWEEN AHH INDUCTION, Ah RECEPTOR BINDING AND TOXICITY

Poland and coworkers have stated "The potency of halogenated aromatic hydrocarbons to induce hepatic AHH activity correlates very closely to their toxic potency" [44] and "that the toxicity of these chlorinated aromatic hydrocarbons is mediated through the receptor; that is the initial event in the toxic action is the stereo-specific recognition and binding of them by the cytosolic binding species" [33]. The evidence for this hypothesis is based on the biologic and toxic effects of several congeneric polychlorinated dibenzo-p-dioxins (PCDDs) and halogenated aromatic compounds which are approximate isostereomers of 2,3,7,8-TCDD [33]. The results which support the proposed correlations are noted below:

1) there is an excellent correlation between the toxic potency of PCDD congeners, their AHH induction activity and affinity for the Ah cytosolic receptor protein (33, for example 2,3,7,8-TCDD is highly toxic, a potent AHH inducer and avidly binds the Ah receptor protein; 2,7,-dichlorodibenzo-p-dioxin is relatively non-toxic, does not bind to the receptor protein or induce microsomal AHH),
2) 2,3,7,8-TCDD and related approximate isostereomers are relatively toxic to the genetically inbred C57BL/6J "responsive mice" and non-toxic to the non-responsive DBA/2J mice [33,42],
3) 2,3,7,8-TCDD and approximate isostereomers induce AHH in the responsive mice however only 2,3,7,8-TCDD is active (at higher concentrations) in the DBA/2J mice [33,44-46].

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PCBs are an ideal group of halogenated aromatic chemicals to test and confirm the correlations proposed by Poland and coworkers. Table 3 illustrates the relative AHH inducing activities 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 and their mono-ortho chloro analogs. The results illustrate that with few exceptions there is an excellent correlation between the relative potencies of the individual PCB congeners as AHH inducers in Wistar rats and rat hepatoma H-4-II-E cells and their avidity for the Ah cytosolic receptor [47]. Although the toxicities of these mono-ortho chlorinated congeners has not been systematically studied there are reports in the literature [16,19,21] which confirm the comparatively high toxicity of the 2,3,3',4,4'-penta-, 2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl. Current research in my laboratory [48,49] with genetically

Table III. PCBs - Effects of Structure on Activity

PCB Congeners (number)	Relative Per Cent Activity		
	AHH Induction		
	Cell Cultures	Rats	Receptor Binding
MC-Type Inducers (3)	100-1	+++	100-35
Mixed-Type Inducers (8)	$0.3\text{-}2.4\times10^{-5}$	++	6-1.5
PB-Type Inducers (4)	inactive	inactive	$\leq$ 0.3
Non-Inducers (3)	inactive	inactive	$\leq$ 0.3
2,3,7,8-TCDD	400	+++++	2,500

inbred mice indicates that administration of the mixed-type PCB inducers to the responsive C57BL/6J mice results in AHH induction and thymic atrophy whereas these compounds do not induce AHH nor are they toxic in the non-responsive DBA/2J mice.

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The results obtained for the PCBs complement the data reported for several PCDD congeners and support the correlations noted by Poland and coworkers [33,34]. Moreover, it is also apparent that PCB congeners which induce AHH activity are toxic and the residue levels of these specific congeners in fish wildlife and exposed humans may be important indicators of potential short and long term toxicity.

PCBs IN HUMANS: PRELIMINARY STUDIES

Figure 3 summarizes all the PCBs expected to exhibit MC-type activity based on the above guidelines and a number of these isomers and congeners have been identified in commercial PCBs and in humans (blood, adipose tissue and breast milk) [50-52]. As noted in Table IV there is relatively high concentration of MC-type inducers which preferentially bioconcentrate in human breast milk. A recent study in our laboratory has shown that the dose effecting half-maximal (ED_{50}) induction of AHH activity for a reconstituted breast milk PCB sample was 12 $\mu mol \cdot kg^{-1}$ 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 and elicit various toxic responses in the rat, chicken and responsive C57BL/6J mice [16,19,21,48,49].

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Table IV. Breast Milk and Reconstituted Breast Milk PCBs [50-53]

PCB Structure	GC Purity (%)	PCB Concentration in Japanese Breast Milk (%)	PCB Concentration in Reconstituted Breast Milk Mixture (%)
2,4,4'-Trichlorobiphenyl	98.5	8.4 ± 2.2	9.2
2,2',5,5'-Tetrachlorobiphenyl	99.0	2.0 ± 1.3	2.2
2,4,4',5-Tetrachlorobiphenyl	99.0	19.1 ± 2.6	20.1
2,2',4,5,5'-Pentachlorobiphenyl	98.5	2.8 ± 0.9	3.0
2,3',4,4',5-Pentachlorobiphenyl	99.0	11.8 ± 1.2	12.8
2,2',3,4',5,5'-Hexachlorobiphenyl	99.0	2.3 ± 0.3	-
2,3,3',4,4'-Pentachlorobiphenyl	99.0	3.5 ± 0.5	3.9
2,2',4,4',5,5'-Hexachlorobiphenyl	99.0	15.5 ± 0.4	16.3
2,2',3,4,4',5'-Hexachlorobiphenyl	99.0	15.8 ± 0.7	16.9
2,2',3,4',5,5',6-Heptachlorobiphenyl	98.5	3.2 ± 0.6	3.3
2,2',3,4,4',5',6-Heptachlorobiphenyl	99.0	1.6 ± 0.3	1.7
2,3,3',4,4',5-Hexachlorobiphenyl	99.0	2.1 ± 0.7	2.3
2,2',3,4,4',5,5'-Heptachlorobiphenyl	90.0	5.1 ± 0.7	5.7
2,2',3,3',4,4',5-Heptachlorobiphenyl	98.5	2.3 ± 0.3	2.3
Total		95.5	99.9

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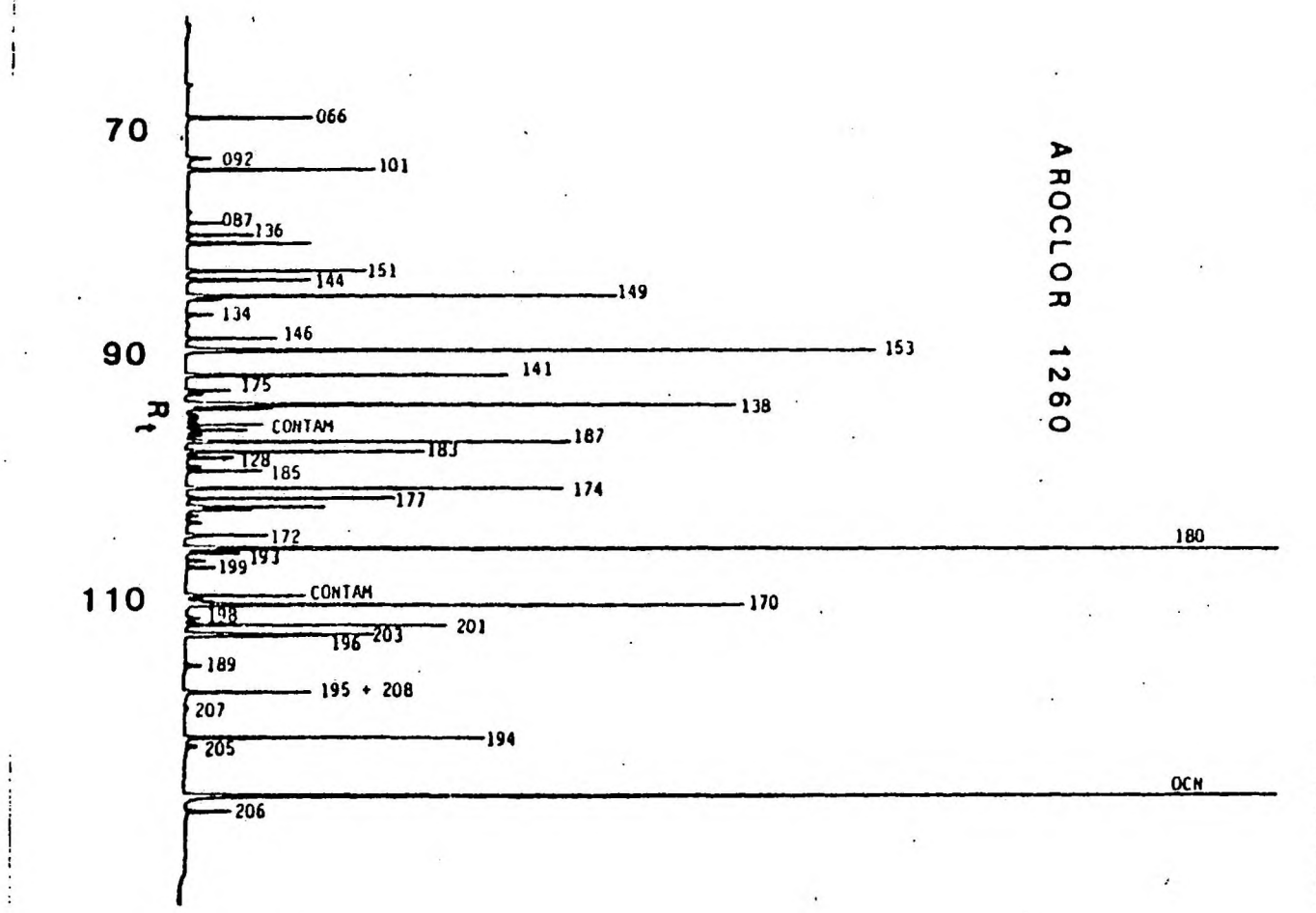

Figure 1. High resolution glass capillary gas chromatogram of Aroclor 1260

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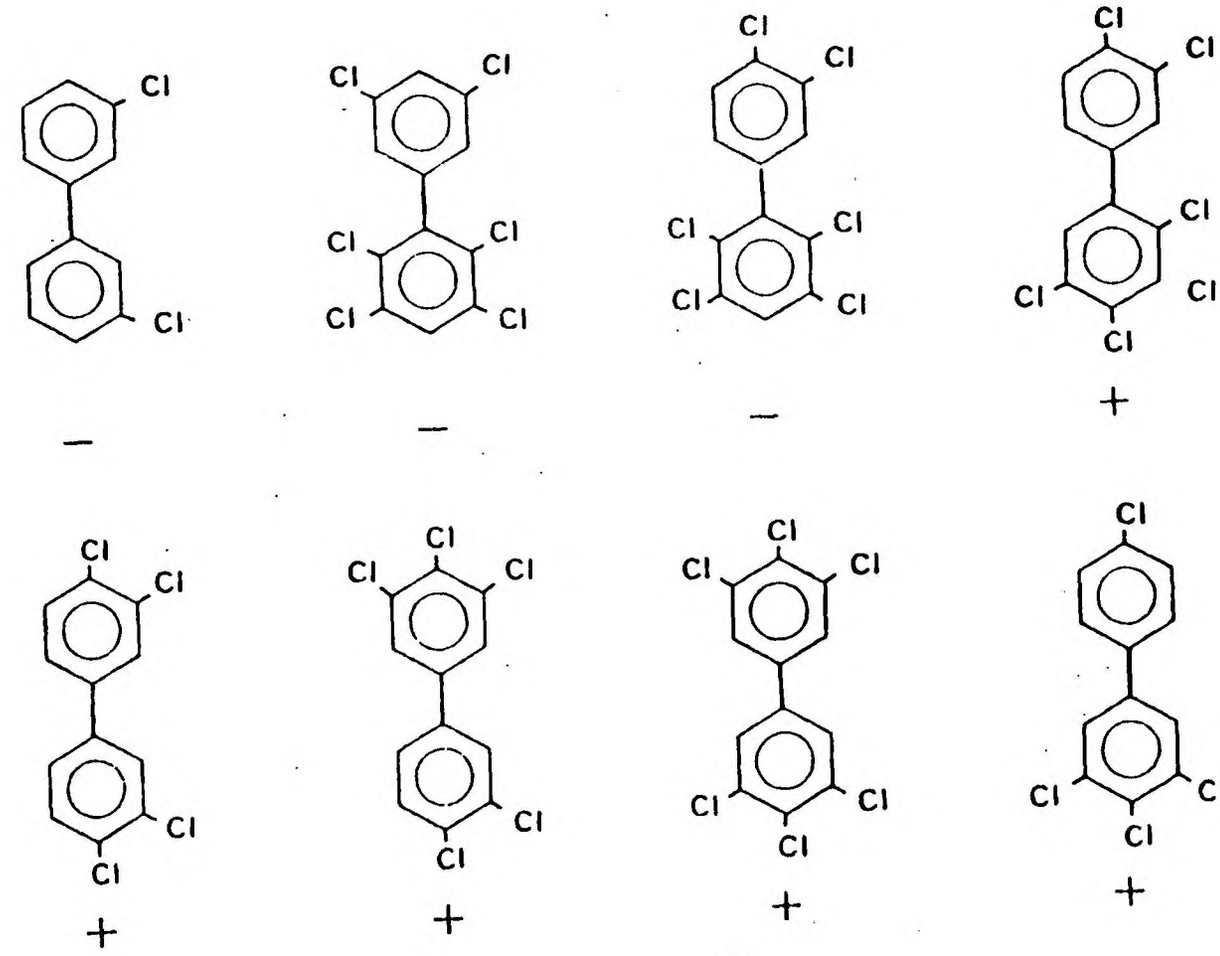

Figure 2. Effects of <u>para</u> and <u>meta</u> substituents on the activity of PCBs as AHH inducers; active (+), inactive (-).

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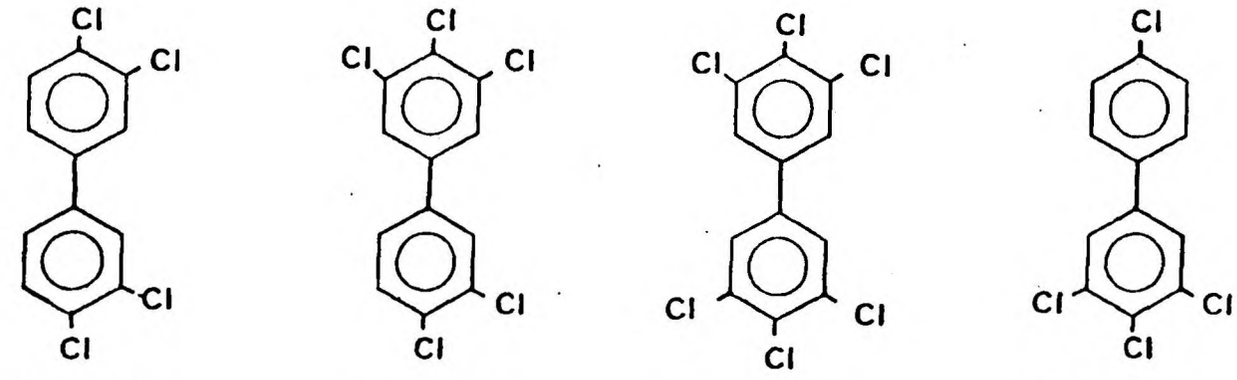

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2 3 3'4 4'6	2 3 3'4 4'5'6		

Figure 3. Mono and 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.

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