

STRUCTURE-ACTIVITY RELATIONSHIPS IN PCBs: USE OF PRINCIPAL COMPONENTS  
ANALYSIS TO PREDICT INDUCERS OF MIXED-FUNCTION OXIDASE ACTIVITY

Joan U. Clarke

Environmental Laboratory  
U.S. Army Engineer Waterways Experiment Station  
Vicksburg, Mississippi 39180

and

Marine Environmental Sciences Consortium  
Dauphin Island, Alabama 36528

Abstract

Of the 209 PCB congeners, over 50 are known or suspected inducers of mixed-function oxidase activities in mammalian liver. These include inducers of cytochromes P-448 (MC-type inducers), P-450 (PB-type inducers), both P-448 and P-450 (mixed-type inducers), and one "novel" inducer. To elucidate structure-activity relationships with respect to these inducers, a statistical approach was taken using principal components analysis. Ten principal components (PCs) were extracted from variables coded for the positions of chlorines on the biphenyl. Three of these PCs, including an "ortho" component and a "para" component, were used to graph a three-dimensional space in which patterns of inducers can be clearly recognized. These patterns include five groups of congeners dominated, respectively, by weak PB-type inducers, MC-type inducers, mixed-type inducers, PB-type inducers, and suspected PB-type inducers. The remaining spatial configuration is a large, heterogeneous group composed of inactive congeners and a few "outlier" PB-type inducers. Re-evaluation of these outliers may indicate that inducer activity is less than previously thought. Conversely, congeners not currently known to be inducers, but falling within the spatial boundaries of the five inducer groups, may also turn out to be inducers. The congeners of the suspected PB-type inducer group are predicted to be weak PB-type inducers, and certain congeners having PC configurations similar to the one novel inducer may possibly also be novel inducers.

Introduction

Polychlorinated biphenyls (PCBs) are among the xenobiotic compounds known to induce hepatic microsomal enzyme activity in vertebrates [1-8]. Mixed-function oxidase (MFO) activities induced by PCB congeners have been characterized as: (1) 3-methylcholanthrene- (MC-) type induction of cytochrome P-448 enzymes, (2) phenobarbital- (PB-) type induction of cytochrome P-450 enzymes, and (3) mixed-type induction of both the cytochrome P-448 and P-450 systems. Parkinson and coworkers [4] have also alluded to a single "novel"-type inducer, which does not fit into any of the above classes, among the PCB congeners. MFO systems function in the elimination of lipophilic xenobiotics by converting them to more water-soluble

metabolites [9]. Reactive electrophilic intermediates are produced in this detoxication process. In some circumstances, such as a limitation of a necessary conjugating substrate, these intermediates may elicit various toxic effects in animals. Endocrine and reproductive dysfunction, teratogenesis, skin disorders, wasting syndrome, carcinogenesis, and death have been linked to production of reactive intermediates beyond the capacity of detoxication systems to eliminate them [6-8, 10].

Not all PCBs are inducers of mixed-function oxidase activities. At present, only a fourth of the 209 congeners are known or suspected to be inducers. Most congeners, particularly the lower chlorinated compounds, are probably inactive or relatively ineffective as inducers due to rapid metabolism [11]. Certain structural prerequisites, i.e. chlorine substitution patterns, appear to be necessary, at least for MC-type and mixed-type induction. Steric effects resulting from different substitution patterns apparently also influence uptake and persistence in aquatic organisms [12-13].

Various investigators [3-4, 6] have proposed structure-activity rules for identifying the induction potential of PCBs based on the substitution patterns of known inducers. These rules may be summarized as follows: (1) substitution in both *para* and at least two *meta* positions of the biphenyl results in MC-type induction; (2) addition of one *ortho* chlorine substitution to the patterns in (1) results in mixed-type induction; and (3) substitution in both *para* and at least two *ortho* positions results in PB-type induction.

A number of PCB congeners not empirically determined to be inducers may be classified in theory as PB-type inducers based on rule (3) above. Conversely, about half of the known mixed-type and PB-type inducers are exceptions to rules (2) and (3). Apparently, these rules are not sufficient to explain the differences in MFO activity induction among the PCB congeners, as some workers have previously stated [4, 14]. This paper is an attempt, using the statistical procedure of principal components analysis as a pattern recognition technique, to elucidate the structural requirements for enzyme induction by PCBs.

#### Structure-Activity Relationships

Within the past few years, pattern recognition techniques have been applied to the derivation of structure-activity relationships, i.e. the association between the structural uniqueness within a group of chemicals and the observed physical-chemical and biological effects (activities) of those chemicals [15]. Pattern recognition is particularly amenable to factor analysis methods, such as principal components analysis (PCA). Several investigators have used PCA as a pattern recognition technique with groups of hazardous chemicals [16-19]. Factor analysis can reduce a raw data set consisting of many variables to a smaller set of derived variables having the statistically desirable property of orthogonality (i.e. the derived variables are uncorrelated with each other). These derived variables can then be used to classify compounds according to effects, either by graphical means or by statistical techniques such as cluster analysis or discriminant analysis.

The derived variables can also be used in regression analysis for the development of quantitative structure-activity relationships (QSAR). QSAR refers to the statistical determination of association between chemical concentrations resulting in a defined biochemical or biological endpoint, and physical-chemical properties or molecular descriptors [20]. However, empirical measurements of properties often used in QSAR, such as partition coefficients and aqueous solubilities, are not yet available for most PCB congeners.

Advantages of using molecular structure information such as connectivity indices, as opposed to measurements of physical-chemical properties, in QSAR development were outlined by Burkhard and coworkers [16]. Molecular connectivity indices have been used to quantitate structural differences in groups of heterogeneous compounds [15-16]. The PCBs, however, are a homogeneous group of congeners, which differ structurally from each other only in the number and positions of chlorine atoms on the biphenyl. With such a group, connectivity indices would provide a large amount of redundant information. Thus, the approach taken herein is the representation of structural differences among all 209 congeners by a coded numerical scheme of the chlorine substitution patterns. This structure-based approach to pattern recognition in the PCBs is appealing from the standpoint of completeness and simplicity.

#### Principal Components Analysis

Principal components analysis (PCA) is a data transformation technique that facilitates the search for patterns within a set of variables. The statistical background and techniques are described in many texts and references [21-24]. PCA on a set of  $p$  variables results in a derived set of  $p$  transformed variables that are linear combinations of the original variables. These derived variables, or principal components (PCs), account for the total variance present in the original data set. The first PC is that linear combination that accounts for as much of the total variance as possible. The second PC is that linear combination that explains as much of the remaining variance as possible, subject to the constraint that it is uncorrelated with (orthogonal to) the first PC. Each successive PC accounts for a declining proportion of the total variance, and all PCs are uncorrelated with each other.

A PC is described mathematically by an eigenvalue and an eigenvector, which can be used to compute a score for the PC on each observation in the original data matrix. Thus, the original data set is totally reconstructed as a matrix of derived principal component scores. PCA may be done using either the correlation matrix or the covariance matrix from the original data. When done on a correlation matrix of  $p$  variables, the eigenvalues corresponding to the  $p$  derived components sum to  $p$ . Since the eigenvalues are measures of variance, the proportion of total variance explained by each PC can be found by dividing its eigenvalue by  $p$ . Each eigenvector is an array of correlations (loadings) between its PC and each of the original variables. The original variables that have the highest positive or lowest negative loadings are those variables that contribute the most directly or inversely to the PC. The analyst may often deduce from these particular variables a meaningful descriptive label for the PC. Such labels can unveil in the data set, structure that was not recognizable in the original variables, thus facilitating pattern recognition.

Since the first few eigenvalues explain the highest proportions of variance in the data set, PCA (and factor analysis in general) can often serve as a data reduction technique by reducing the dimensionality of the raw data. Frequently, the first two or three PCs will account for more than 90% of the variation in the data, and subsequent PCs can be discarded while sacrificing little information. This is particularly true when some of the original variables are highly intercorrelated (multicollinear). If the original variables are essentially uncorrelated with each other, then PCA will not contribute much toward data reduction, but may still be quite useful in pattern recognition.

Besides data reduction and pattern recognition, PCA has a third important function. As previously stated, the PCs are, by mathematical definition, uncorrelated with each other. The principal component scores may be used instead of the original data set in any statistical technique, such as multiple regression analysis, discriminant analysis, or cluster analysis. This eliminates the multicollinearity problems often encountered with these techniques when the original, untransformed variables are used.

#### Results of Principal Components Analysis on the PCBs

A data set was constructed to distinguish the 209 PCB congeners in terms of the number and position of chlorine atoms on the biphenyl. Ten dummy variables were created to represent the ten possible chlorine substitution positions. Each variable was assigned a value of 1 if its corresponding position was substituted, and 0 if unsubstituted. PCA was then done on the correlation matrix from these ten positional variables using the Statistical Analysis System (SAS) PRINCOMP procedure, resulting in ten principal components with associated eigenvalues as listed in Table 1. It is apparent that virtually all of the principal

TABLE 1  
Results of Principal Component Analysis

Principal Component	Eigenvalue	Variance Explained	
		Percent	Cumulative Percent
PC1	1.726	17.3	17.3
PC2	1.411	14.1	31.4
PC3	1.368	13.7	45.1
PC4	1.087	10.9	55.9
PC5	1.075	10.8	66.7
PC6	0.961	9.6	76.3
PC7	0.849	8.5	84.8
PC8	0.598	6.0	90.8
PC9	0.532	5.3	96.1
PC10	0.391	3.9	100.0

components are required to account for the variance in the data set, and the proportions of variance explained by the first PCs are not substantially different from those explained by the last. This result is expected since the positional variables are essentially uncorrelated with each other. Thus, the PCA cannot serve in data reduction.

The intent of PCA in the present context is to facilitate pattern recognition. From the eigenvectors, structural diagrams were constructed to represent the relative contribution of each positional variable to each PC. In Figure 1, positive loadings of the positional variables on the PCs are represented by solid lines in the chlorine positions, and negative loadings by dashed lines. The length of the line indicates the relative magnitude of the loading. Loadings greater than -0.2 and less than +0.2 on a scale of -1 to +1 are not illustrated. Structurally meaningful labels for some of the PCs can be deduced from the diagrams. For example, it is immediately obvious that PC1 is an *ortho* component, while PC4 is a *para* component. No corresponding *meta* component exists, although PC3 is characterized by a strong contribution from *meta* substitution along with absence of *ortho* substitution on ring A. *Meta* substitution, especially on ring B, is also important in PC2, along with a lesser contribution from *ortho* substitution on ring A. PC5 is dominated by a *meta* and a *para* substitution on opposite rings.

Principal component scores on the ten PCs were calculated for each of the 209 PCBs. To determine which of the PCs might be associated with the enzyme-inducing properties of PCBs, scatterplots of PC scores were examined for all possible combinations of two PCs. Patterns of inducers were discernible in some combinations involving PCs 1, 4, and 5. These three components were also found to correlate more highly than the other PCs with a ranked variable representing inducer types among the congeners.

Figures 2 and 3 are three-dimensional representations of the 209 congeners on PCs 1, 4, and 5. All congeners are plotted as cross-marks on the PC1 - PC4 plane, but only those known to be inducers and certain others falling near them are illustrated in the three-dimensional space created by adding the PC5 axis. Congeners with positive scores on PC5 rise above the PC1 - PC4 plane and are connected to it by solid lines; congeners with negative scores on PC5 fall below the plane and are connected by dashed lines. Figure 2 differentiates the types of inducers; these same congeners are shown in Figure 3 with their respective Ballschmiter and Zell congener numbers [25]. Most of the inducers fall into fairly discrete groups, indicated by Roman numerals. The rest are considered to be outliers. The groups may be characterized as follows:

- Group I - weak PB-type inducers or inactive
- Group II - primarily MC-type inducers
- Group III - primarily mixed-type inducers
- Group IV - primarily PB-type inducers
- Group V - entirely PB-type inducers.

Several other observations are noteworthy. (1) Groups I, II, and III include congeners not currently known to be inducers. (2) Most congeners of Group IV falling on the positive side of PC1, and all but two congeners in Group V have been classified as "theoretical" PB-type inducers by rule (3) of the proposed structure-activity rules, but have not been empirically investigated for induction ability. (3) The one known "novel" inducer is the only inducer in Group III with a substantial negative score (-0.5) on PC5. (4) The outlying inducers are scattered and appear to have little in common structurally. (5) Most of the inducers have

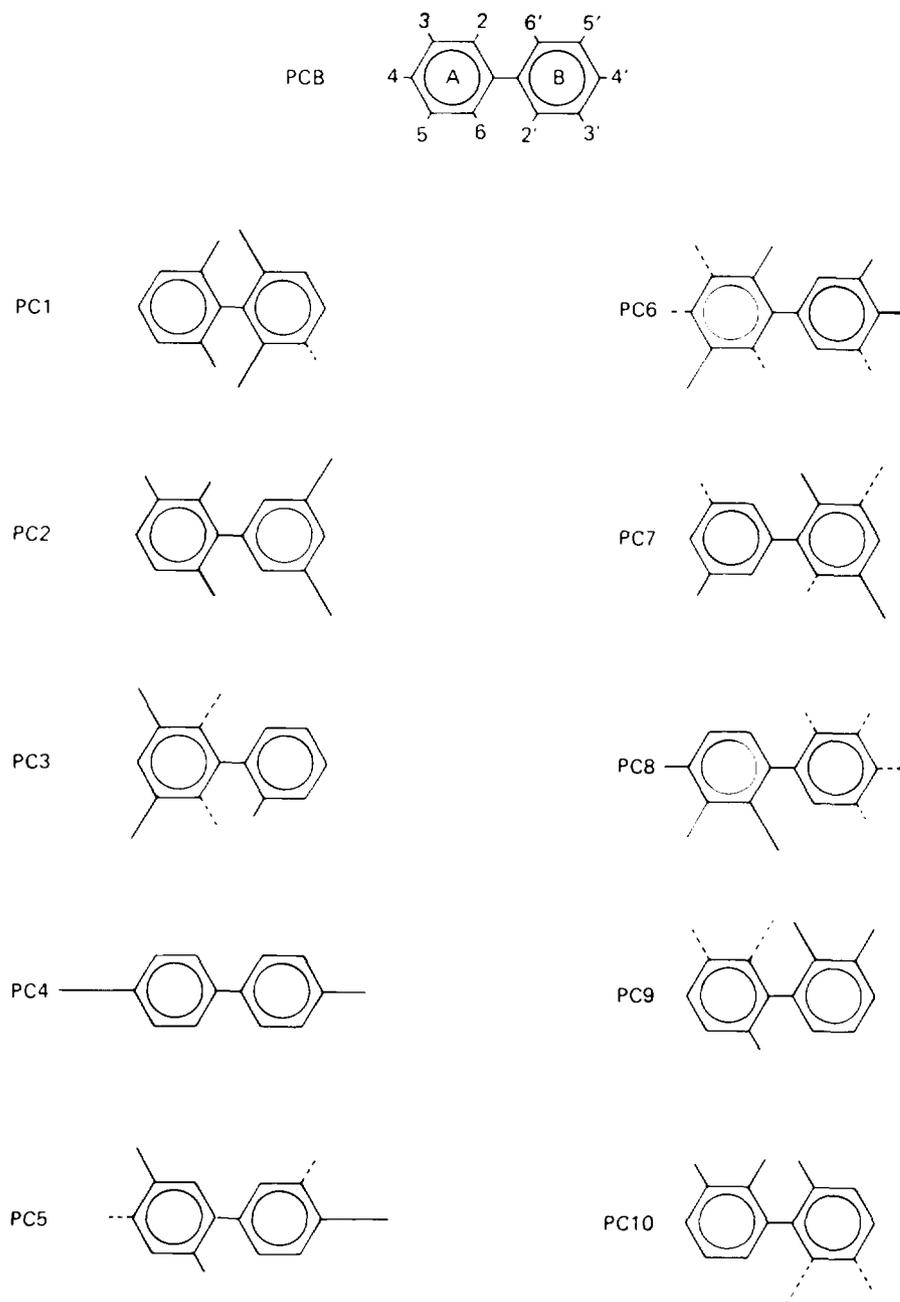


Figure 1. Schematic diagrams of PCB showing the numbered chlorine positions, and the relative contributions of chlorine positional variables to each principal component.

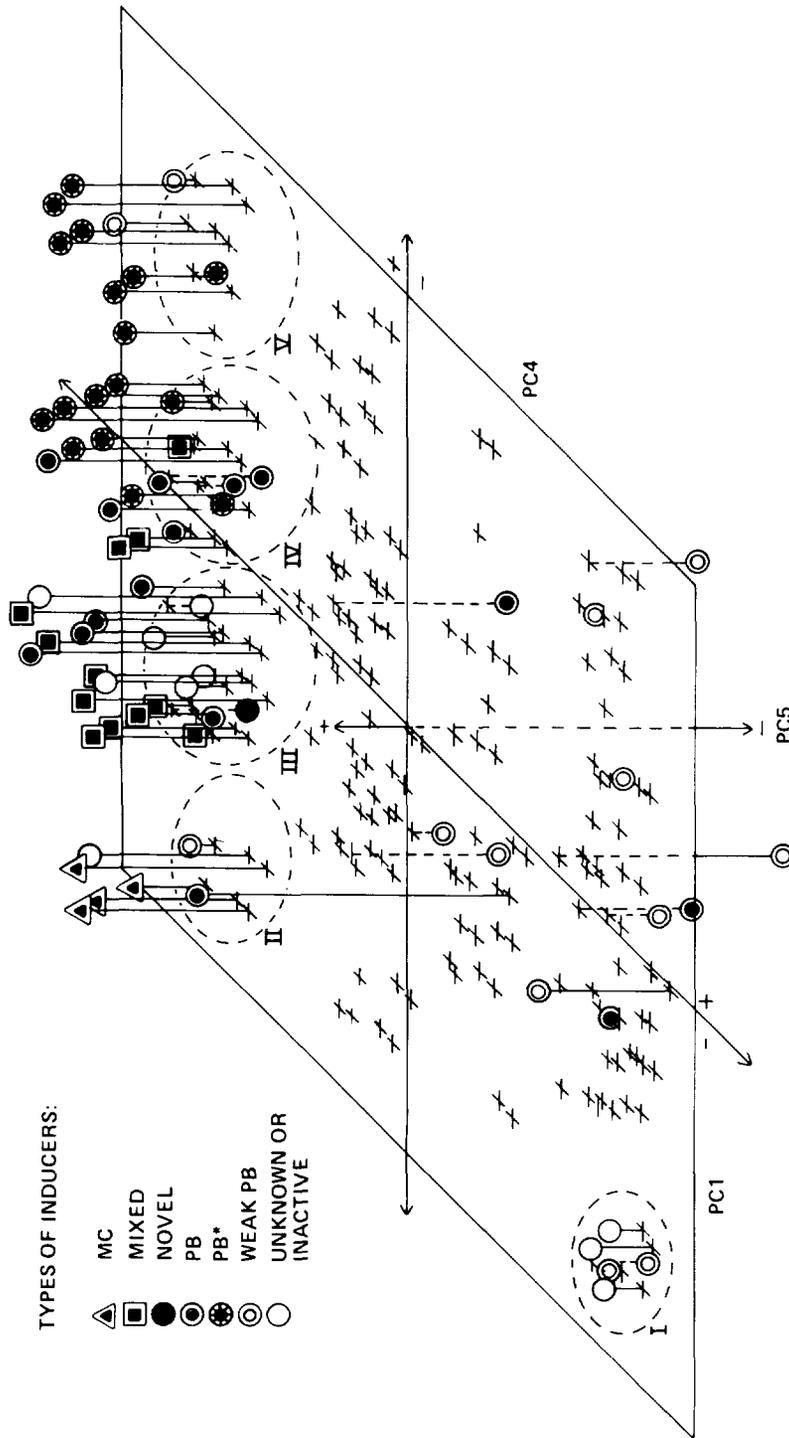


Figure 2. Three-dimensional representation, by inducer type, of PCB congeners having known or potential MFO induction properties. Axes are principal components 1, 4, and 5.



positive scores on PC4 and PC5. (6) PCs 4 and 5 tend to separate inducers from non-inducers, whereas PC1 functions more to differentiate among the types of inducers.

### Discussion

The congeners shown in Figures 2 and 3 are described in Table 2. Group I comprises all congeners having *meta* substitution only. Two of the five congeners in this group have been described as weak PB-type inducers, but they, along with most of the other weak inducers, probably have little biological significance.

Group II contains all congeners having both *para* positions substituted and no *ortho* substitution, and includes the four MC-type inducers. The other two congeners are a dichlorobiphenyl and a trichlorobiphenyl. Most lower chlorinated congeners, regardless of structure, are probably inactive or relatively ineffective as inducers due to rapid metabolism [3, 6, 8].

Congeners in Group III all have two *para* chlorine substitutions and one or two *ortho* substitutions. This group includes several congeners that have not been reported to have enzyme inducing ability. On a structural basis these might be suspected of being mixed-type or PB-type inducers. Only one congener in the group, No. 168, has been categorized as inactive [4]. Group III also contains the only known novel inducer, No. 167. Other congeners in Groups III and IV also have negative scores on PC5; these include Nos. 66, 168, 99, 47, and 153. On the basis of the PCA, these may be suspected of having induction properties similar to No. 167.

Groups IV and V are characterized by the continued presence of two *para* chlorines and an increasing number of *ortho* substitutions. The general trend as scores on PC1 increase (i.e. as number of *ortho* substitutions increases) across Groups II, III, IV, and V, appears to be a decline in inducer potency from MC-type to mixed-type to strong PB-type to theoretical PB-type and weak PB-type inducers. Many of the theoretical PB-type inducers in Groups IV and V may well be weak or ineffective inducers. These are the highest molecular weight PCBs (hepta- through decachlorobiphenyls), which may encounter steric hindrance to passage through membranes [33]. The mixed-type inducers of Group IV may possibly be PB-type inducers instead. Goldstein [11] suggested that the cytochrome P-448 induction attributed to Nos. 128 and 138 may reflect trace contamination by a chlorinated dibenzofuran such as the potent MC-type inducer 2,3,7,8-tetrachlorodibenzofuran (TCDF). If such is the case, then these congeners, which have 2,2'-*ortho* substitution, would conform more closely to the structure-activity rules cited earlier, and would contribute to more homogeneous PCA groups as well.

All of the outliers have been reported as PB-type inducers. Most of these congeners have been characterized as weak or relatively ineffective in their induction capability. Four of the outliers, Nos. 87, 133, 163, and 165, are thought to be moderate or strong PB-type inducers [2, 6, 14, 31]. It may be that induction activity exhibited by these structurally diverse outliers was due to metabolites or to trace contamination by potent inducers such as TCDF. The results of the PCA would suggest that the outliers be reinvestigated for induction activity.

TABLE 2  
Description of Inducers and Other PCB Congeners Selected by  
Principal Components Analysis Pattern Recognition Technique

Group	Congener No. <sup>1</sup>	Structure	Type of Inducer <sup>2</sup>	References
I	2	3	-	
	11	3,3'	weak PB	6,8,11,14,26
	14	3,5	-	
	36	3,3',5	-	
	80	3,3',5,5'	weak PB or IA	2,8,11,27
II	15	4,4'	weak PB	6,8,11,14,26
	37	3,4,4'	-	
	77	3,3',4,4'	MC	2,3,6,8,11,14,27-30
	81	3,4,4',5	MC	6,14
	126	3,3',4,4',5	MC	3,6,14,29
	169	3,3',4,4',5,5'	MC	2,3,6,8,11,14,27-31
III	28	2,4,4'	-	
	60	2,3,4,4'	-	
	66	2,3',4,4'	PB	14
	74	2,4,4',5	-	
	75	2,4,4',6	PB	26
	105	2,3,3',4,4'	mixed	3,6,14
	114	2,3,4,4',5	mixed	6,14
	115	2,3,4,4',6	-	
	118	2,3',4,4',5	mixed	2,3,6,14
	119	2,3',4,4',6	-	
	123	2',3,4,4',5	mixed	6,14
	156	2,3,3',4,4',5	mixed	3,6,14
	157	2,3,3',4,4',5'	mixed	3,6,14
	158	2,3,3',4,4',6	mixed	4,6
	166	2,3,4,4',5,6	mixed	4,6
	167	2,3',4,4',5,5'	novel	4
	168	2,3',4,4',5',6	IA	4
	189	2,3,3',4,4',5,5'	mixed	2,3,6,14,30
	190	2,3,3',4,4',5,6	PB	4
	191	2,3,3',4,4',5',6	PB	4
205	2,3,3',4,4',5,5',6	PB	4	
IV	47	2,2',4,4'	strong PB	2,3,8,11
	85	2,2',3,4,4'	PB*	
	99	2,2',4,4',5	PB*	
	100	2,2',4,4',6	PB*	
	128	2,2',3,3',4,4'	mixed	4,6,32
	137	2,2',3,4,4',5	PB	4
	138	2,2',3,4,4',5'	mixed	4,6,32-33
	139	2,2',3,4,4',6	PB*	
	153	2,2',4,4',5,5'	strong PB	2-4,6,8,11,30-32
	170	2,2',3,3',4,4',5	mixed	4,6
	171	2,2',3,3',4,4',6	PB*	
	180	2,2',3,4,4',5,5'	PB	4
	181	2,2',3,4,4',5,6	PB*	
	183	2,2',3,4,4',5',6	PB*	
	194	2,2',3,3',4,4',5,5'	strong PB	4,11
	195	2,2',3,3',4,4',5,6	PB	4
	203	2,2',3,4,4',5,5',6	PB*	
206	2,2',3,3',4,4',5,5',6	PB*		
V	140	2,2',3,4,4',6'	PB*	
	154	2,2',4,4',5,6'	PB*	
	155	2,2',4,4',6,6'	weak PB	2,8,11
	182	2,2',3,4,4',5,6'	PB*	
	184	2,2',3,4,4',6,6'	PB*	
	196	2,2',3,3',4,4',5,6'	PB*	

TABLE 2 (Concluded)

Group	Congener No. <sup>1</sup>	Structure	Type of Inducer <sup>2</sup>	References
V (contd.)	197	2,2',3,3',4,4',6,6'	PB*	
	204	2,2',3,4,4',5,6,6'	PB*	
	207	2,2',3,3',4,4',5,6,6'	PB*	
	209	2,2',3,3',4,4',5,5',6,6'	weak PB	32,33
Outliers	40	2,2',3,3'	weak PB or IA	8,32
	52	2,2',5,5'	weak PB or IA	2,32
	54	2,2',6,6'	weak PB or IA	2,8,31,32
	61	2,3,4,5	weak PB or IA	8
	65	2,3,5,6	weak PB or IA	8
	87	2,2',3,4,5'	PB	2
	133	2,2',3,3',5,5'	strong PB	2,6,27,32
	136	2,2',3,3',6,6'	weak PB or IA	2,8,32
	151	2,2',3,5,5',6	weak PB	6,29
	159	2,3,3',4,5,5'	weak PB or IA	29
	163	2,3,3',4',5,6	PB	6,14
	165	2,3,3',5,5',6	PB	6,14

<sup>1</sup> From Ballschmitter and Zell [25].

<sup>2</sup> - Unknown; IA Inactive or relatively ineffective; PB\* Theoretical PB-type inducer from rule (3).

Besides facilitating pattern recognition, PCA provides a set of orthogonal derived variables that contain all the information present in the original data set and can be used in any subsequent analysis. A logical extension of the PCA herein would be the use of the PCs for the derivation of QSAR relating physical-chemical properties to structure. The PCs could also be applied to bioaccumulation data in an attempt to elucidate structural features associated with the preferential uptake of some PCB congeners over others. This application will become useful as improved analytical techniques make it possible for investigators to quantitate individually a large number, preferably all, of the 209 congeners. In cases where quantitation of all congeners is not feasible, the PCA has defined a subset of congeners (those in Groups II-V and the outlying inducers) that are most likely to be of toxicological importance.

### Summary

Principal components analysis serves as a useful pattern recognition technique in the characterization of known MFO inducers and the search for potential inducers among the PCB congeners. If the 209 congeners are described by a set of variables coding for chlorine substitution, PCA on these variables can extract relevant structure-activity information.

The implications of the PCA conducted herein are that: (1) congeners not currently known to be inducers but falling within the inducer groups, particularly Group III, should be investigated for induction potential; (2) the outlying inducers should be reexamined because structurally they are quite different from congeners in the inducer groups, and thus their induction activity may be less than previously thought; (3) if other novel inducers exist besides No. 167, likely candidates, based on their PC scores, would be Nos. 66, 168, 99, 47, and 153; and (4) congeners designated as theoretical PB-type inducers from previously proposed

structure-activity rules are most likely to be moderate or weak inducers based on their structural resemblance to known inducers falling within Groups IV and V.

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