A Resource for PCB Environmental Chemistry and Biology: Antibody Affinity Values for the Entire Set of 209 PCB Congeners

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INTRODUCTION

PolyChlorinated Biphenyls (PCBs) are a mixture of the 209 mono- to decachlorinated isomers of biphenyl (1). PCBs are ubiquitous in the environment (2). The PCB isomers (congeners) which are currently believed to be the most environmentally significant are ortho unsubstituted and coplanar. They occur in much smaller amounts than the dominant, less toxic congeners in industrial PCB formulations and environmental samples (1,2). As a result of the growing recognition that congener specific PCB analysis is required for an objective evaluation of risk and environmental impact (3,4), we developed an Enzyme ImmunoAssay (EIA) which is specific for the most toxic, coplanar PCB congeners (5): PCB 77, 3,3',4,4'-tetrachlorobiphenyl; PCB 126, 3,3',4,4',5-pentachlorobiphenyl; PCB 169, 3,3',4,4',5,5'-hexachlorobiphenyl (5). This assay was used in the development and application of procedures for the analysis of selected congeners in complex PCB mixtures (6).

Evaluation of PCB contamination of the environment is critical to our assessment of PCB environmental impact. However, defining the role of PCBs in the environment and developing methods for reduction of their environmental impact requires an understanding of PCB toxicology at the molecular level. Specifically, what is the quantitative relationship between protein (antibody, Ah receptor, Cytochrome P_{540}, etc.) structure and PCB congener binding? The anti-PCB antibody which was used in the development of the coplanar congener EIA (MAb S2B1.9 (5)) is a resource for the study of the PCB congeners and their protein/receptor interactions. This study takes the first step toward the development of PCB congener / receptor interaction models through determination of the cross-reactivity (and hence antibody binding constant ($K_{binding}$) for all 209 PCB congeners versus the S2B1.9 antibody. Preliminary applications of this data set and suggested directions for future studies will be discussed.

MATERIALS AND METHODS

The reported EIA was based on a specific monoclonal antibody (S2B1.9) and competitor conjugate combination (5). These reagents have been formatted for use in a coated tube method (6). The various congeners were purchased as a complete set from Accustandard, Inc. (New Haven, CT, USA) and prepared as serial dilutions in methanol which contained 200 ppb Triton X-100 detergent. All congeners were evaluated for antibody binding using the EIA at 500,000 pg per assay tube. All congeners which gave a response in the assay at this concentration were further evaluated using multiple standard curves and appropriate dilutions ranging from 1 - 500,000 picograms per tube. Cross-reactivities are defined on a weight/weight basis using the assay equilibrium point (I_{50}) for PCB 126 of 110 picograms/tube.

RESULTS AND DISCUSSION

This data resource is currently under development. The following examples illustrate the directions which are being pursued to develop a quantitative model of the interaction of the PCB

congeners and the antibody combining site.

1) Antibody Affinity. Cross-reactivities for this data set were defined using PCB 126 (3,3',4,4',5-pentaCB) as the reference with a cross-reactivity value of 1.00. The range of cross-reactivities for the 209 congeners were from <0.000022 to 1.00 (data not shown). S2B1.9 antibody affinity for PCB 126 can be determined from the immunoassay standard curve data (7). This analysis gives a $K_{binding}$ for PCB 126 to S2B1.9 of 1.63×10^9. Based on this value, the range of $K_{binding}$ for the 209 congeners is $<3.6 \times 10^4$ to 1.63×10^9. Clearly, this 5 decade range of binding affinity provides sufficient scope for a study of the effects of both antibody and PCB congener structure on binding.

2) Relationship of Congener Structure to Antibody Binding. Figure 1 presents the congener cross-reactivity data as a Congener Number versus Log Cross-Reactivity bar chart. Only 11 of the 209 congeners bind to the antibody with affinities which are within an order of magnitude of PCB 126 [cross-reactivities of 0.10 - 1.00]. These congeners are: [PCB# - chlorine substitution (cross-reactivity)] 35 - 34/3-tri (0.13); 77 - 34/34-tetra (0.63); 78 - 345/3-tetra (0.85); 79 - 34/35-tetra (0.79); 124 - 345/24-penta (0.21); 126 - 345/34-penta (1.00); 127 - 345/35-penta (0.98); 159 - 2345/35-hexa (0.21); 162 - 235/345-hexa (0.17); 169 - 345/345-hexa (0.40); 189 - 2345/345-hepta (0.17). This pattern of high affinity ($K_{binding} > 10^8$) congeners confirms the empirical expectation that the antibody binding pocket resembles the 3,3',4,4'-substituted biphenyl hapten which was used to elicit this antibody. On a more detailed basis, structural connectivity can be a significant source of structure / binding information. As listed above, the relative cross-reactivity of PCB 126 (345/34) is 1.00 and that of PCB 78 (loss of the 4'-chlorine to give 345/3) is 0.85. The cross-reactivity of PCB 81 (loss of the 3'-chlorine to give 345/4) is 0.0047. Therefore, loss of the 4'-chlorine results in only a 1.2-fold loss of cross-reactivity versus PCB 126 while loss of the 3'-chlorine results in a 210-fold loss of cross-reactivity. Clearly, significant information can be derived from a detailed examination of the relationship between structural connectivity and antibody binding.

3) Relationship of Congener Physical Properties to Antibody Binding. A substantial data base of PCB congener properties have been measured or derived, e.g., octanol-water partition coefficient (8), torsional angle (9), etc. However, preliminary evaluation of the relationship between antibody binding and these properties demonstrates that, as expected, correlation is not based on any single property. For example, Figure 2 shows the plot of Log Cross-Reactivity versus Log Kow for all 209 congeners. There was no correlation for first through third order line fits of this data (R^2 0.15-0.16). Multiple property correlations will be pursued as the next phase of this part of the study.

4) Relationship of Antibody Structure to Antibody Binding. The antibody S2B1.9 is currently being cloned for derivation of the gene and amino acid sequences of the heavy and light chain combining site regions. This data will be invaluable in the development of the final PCB congener / antibody binding site models.

CONCLUSION

Clearly, the most effective use of this 209 congener data set will require a comprehensive synthesis of all of the above approaches and their variables. These studies are in progress.

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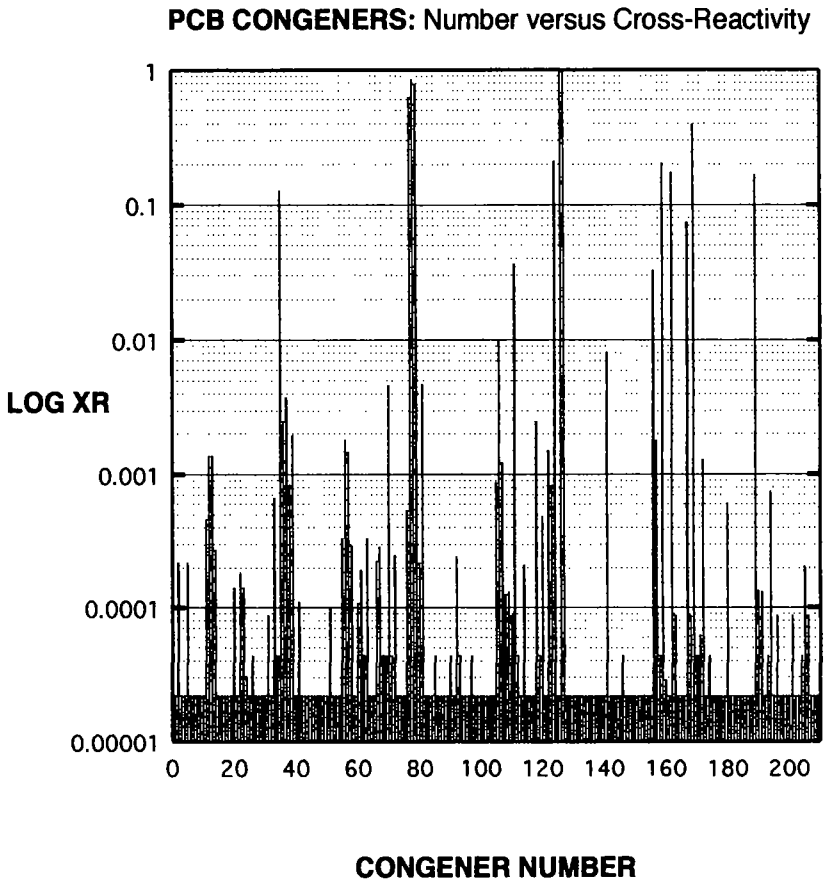

FIGURE 1. BAR CHART PLOT OF PCB CONGENER NUMBER VERSUS LOG CROSS-REACTIVITY. The <0.000022 cross-reactivity values were plotted as 0.000022.

FIGURE 2. CORRELATION PLOT OF LOG CROSS-REACTIVITY VERSUS LOG PARTITION COEFFICIENT (Kow). The <0.000022 cross-reactivity values were plotted as 0.000022.