A TOXIC CONGENER SPECIFIC, MONOCLONAL ANTIBODY-BASED IMMUNOASSAY FOR PCBs

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INTRODUCTION

The most toxic PCB congeners are ortho unsubstituted and coplanar. They occur in much smaller amounts than the less toxic congeners in industrial PCB formulations and environmental samples. There is growing recognition that specific analysis of the toxic PCB congeners in the environment is required for an objective evaluation of risk and environmental impact.[1] However, the time, effort and expense associated with the congener specific analysis of these compounds by instrumental methods such as capillary gas chromatography places substantial constraints on the scope of risk assessment and site evaluation studies. Immunoassay based analytical methods have demonstrated value for specific, high throughput screening as well as quantitative analyses of many environmental analytes.[2] We have developed direct enzyme immunoassays (EIAs) which are specific for the most toxic, coplanar PCB congeners. These EIAs use a monoclonal antibody which is derived from a coplanar hapten and a novel, PCB fragment derived competitor which is used in the preparation of an enzyme conjugate. The assay can be completed in less than 30 minutes. The coated-tube EIA has a minimum detection limit of less than 0.2 ppb and an I_{50} of less than 1 ppb for the 3,3',4,4'-tetrachloro and 3,3',4,4',5-pentachlorobiphenyl congeners. Cross-reaction with several of the less toxic but more common Aroclor congeners including 4,4'-dichloro-, 2,2',5,5'-tetrachloro- and 2,2',4,4',5,5'-hexachlorobiphenyl is less than 0.01%. This presentation will describe the development and characterization of this assay.

MATERIALS AND METHODS

Experimental details of the hapten and competitor synthesis, MAb derivation and assay characterization will be reported elsewhere (Y-W. Chiu et al. in preparation).

Hapten and Competitor Synthesis and Conjugation. Hapten synthesis utilized coupling of 3,4-dichloroaniline with 2-chloroanisole to produce a methoxytrichlorobiphenyl intermediate which was converted to the hapten. Competitor synthesis was based on the addition of a nucleophilic chlorophenyl species to an electrophilic linker synthon. Bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH) and horseradish peroxidase (HRP) conjugates were prepared using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide / n-hydroxysuccinimide activation of the hapten or competitor pendant carboxyl group.

Antisera and Monoclonal Antibody Development. Mouse immunization and monoclonal antibody preparation followed the procedures described in Karu et al.[3]

Immunoassay. The EIAs used for antibody evaluation and assay development made use of standard reagents and procedures. The indirect EIA (immobilized competitor conjugate) was performed essentially as described previously.[3] Direct EIAs (immobilized antibody, competitor-enzyme conjugate) were done according to standard EnviroGard test kit procedures (Millipore Corp., Bedford, MA). A direct EIA was also devised using competitor-enzyme conjugate and covalently immobilized streptavidin to capture biotinylated MAb S2B1 (Y-W. Chiu et al. in preparation). This assay was used for the cross-reactivity studies because it was very stable with assay diluents containing up to 20% methanol, DMSO or acetonitrile.

RESULTS AND DISCUSSION

Hapten and Competitor Design and Preparation. Hapten design was based on 3,3',4,4'- substitution because the non-ortho substituted, coplanar PCB congeners (3,3',4,4'-tetrachloro-, 3,3',4,4',5-pentachloro- and 3,3',4,4',5,5'-hexachlorobiphenyl) are the most toxic based on numerous criteria including their binding to the aryl hydrocarbon receptor.[4] Substitution of an ether linker as a chlorine mimic and placement of the linker moiety at the least sterically sensitive para position[5] led to the design and synthesis of Hapten I (**1**, Figure 1). Our development of an Aroclor directed immunoassay demonstrated that PCB fragment based competitors would produce the most sensitive assays.[5] Consequently, we hypothesized that various combinations of chlorine substitution pattern and linker functional group on a phenyl core would have different effects on assay sensitivity. The competitors illustrated in Figure 1 (**2-5**) were evaluated in this study.

Polyclonal Antiserum Evaluation. Based on our previous observation that different mouse strains may respond differently to immunizing haptens, four mice each of the Swiss Webster, Biozzi and B10.Q strains were immunized with the Hapten-I KLH conjugate. Sera taken from three of the mice about 110 days after the initial dose showed competitive binding of both 3,3',4,4'-tetrachloro- and 3,3',4,4',5-pentachlorobiphenyl in both the direct and indirect EIAs using the 2,5-S competitor (**4**, Figure 1). Serum from the best mouse (Swiss Webster no. 2007-1) was further evaluated by comparing the competitors (Figure 1) to the hapten in the direct EIA using 3,3',4,4'-tetrachlorobiphenyl as the analyte (Table I). These results demonstrated that assays based on the PCB fragment derived competitors were 5- to 27-fold more sensitive than the homologous (hapten based) assay.

TABLE I. Comparison of competitor derived 3,3',4,4'-tetrachlorobiphenyl EIA sensitivities using mouse 2007-1 serum.

COMPETITOR	I_{50} (ppb)
Hapten I (**1**)	30-40
2,5-CH2 (**2**)	6-8
3,4-O (**3**)	3.5
3,4-keto (**5**)	3
2,5-S (**4**)	1.5

Direct and indirect EIAs using 2007-1 serum and the 2,5-S competitor (**4**, Figure 1;

data not shown) had a low ppb I_{50} for 3,3',4,4'-tetrachlorobiphenyl, with approximately 10% cross-reaction with 3,3',4,4',5-pentachlorobiphenyl. The assays gave less than 1% cross-reaction with 2,2',4,4',5,5'-hexachlorobiphenyl and related ortho substituted congeners which are abundant but relatively non-toxic Aroclor constituents. Aroclor 1248 gave an assay response corresponding to about 2 mole percent of 3,3',4,4'-tetrachlorobiphenyl. This compares well with a calculated co-planar, toxic congener content of about 1% for this Aroclor.[6] These results provide an additional indication that the assay did not significantly respond to the more abundant non-coplanar Aroclor congeners.

Monoclonal Antibody Derivation. The antisera results demonstrated that a toxic congener-specific PCB assay was feasible, and indicated that monoclonal antibodies derived from mouse 2007-1 might provide better congener discrimination than a polyclonal serum-based assay. The preparation of hybridomas followed our standard procedures.[3] A total of 284 hybridomas produced antibodies that bound BSA conjugates of Hapten-I or 2,5-S (**4**, Figure 1). However, only one hybridoma line, designated S2B1, produced a MAb with the desired sensitivity and specificity for the coplanar PCB congeners.

Assay Characterization. Figure 2 shows the selective binding of the toxic PCB congeners by MAb S2B1 in a direct competition EIA using the 3,4-keto competitor (**5**, Figure 1). The microplate EIA had an I_{50} of approximately 3 ppb for 3,3',4,4'-tetrachlorobiphenyl and 3,3',4,4',5-pentachlorobiphenyl (Figure 2). An optimized coated-tube EIA had a minimum detection limit of 0.2 ppb and an I_{50} of 1 ppb for these target congeners. Binding of the third toxic, coplanar congener, 3,3',4,4',5,5'-hexachlorobiphenyl, was very dependent on the concentration and type of organic solvent in the assay diluent. Virtually no inhibition by up to 1 ppm of the hexachloro congener was observed in diluent with 5% methanol, and only 15 to 25% inhibition occurred in diluent with 10% or 20% methanol. However, 5 ppm of this congener gave up to 80% inhibition in diluent containing 10% or 20% DMSO. In contrast, no binding was observed with up to 1 ppm of any of the non-coplanar congeners, regardless of the diluent solvent or its concentration.

Table II summarizes the cross-reactivity of the direct EIA using MAb S2B1 and the 3,4-keto competitor (**5**, Figure 1). Several of the most abundant ortho substituted congeners were not recognized by this assay. The assay also did not cross-react significantly with single-ring chlorinated compounds or the toxic hydroxylated mammalian PCB metabolites. Perhaps most importantly, the <1% cross-reaction with 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran makes this EIA useful for discrimination of the toxic PCBs from these related, aryl hydrocarbon receptor active environmental contaminants.

CONCLUSIONS

1. A sensitive, congener specific EIA was developed using a coplanar, ether-linked hapten, a monoclonal antibody derived from the hapten and PCB fragment derived competitors.
2. The assay discriminates between the most toxic coplanar PCBs and the more abundant, less toxic congeners in commercial PCB formulations.
3. The assay does not respond to other common chloroaromatic environmental

contaminants, especially 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran.

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FIGURE 1. Hapten and Competitor Structures.

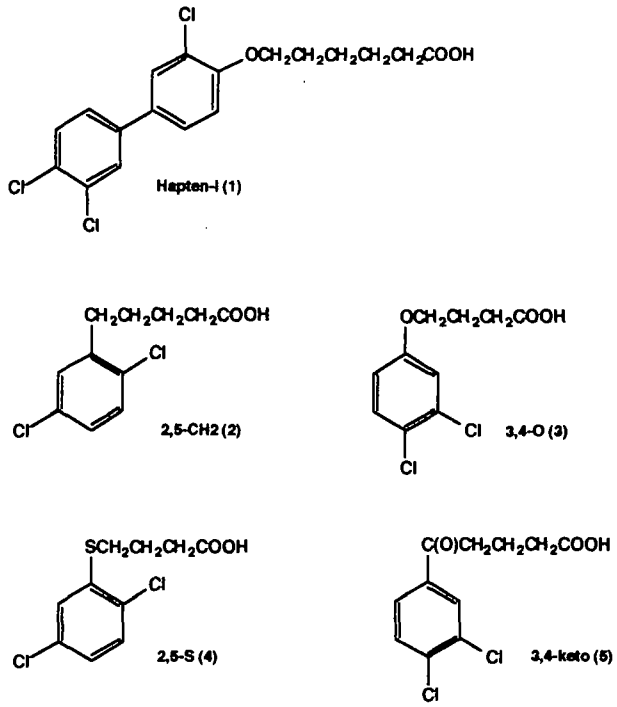

FIGURE 2. Direct competition EIA of six PCB congeners with MAb S2B1. (✤), 2,4′-dichlorobiphenyl; (✣), 2,5,2′,5′-tetrachlorobiphenyl; (▲), 2,4,5,2′,4′,5′-hexachlorobiphenyl; (△), 2,4,4′-trichlorobiphenyl; (—●—), 3,4,3′,4′-tetrachlorobiphenyl; (--○--), 3,4,3′,4′,5′-pentachlorobiphenyl. The lines are 4-parameter logistic fits of the data. The estimated I_{50} values were 0.9 and 1.2 ppb for 3,4,3′,4′-tetra- and 3,4,3′,4′,5′-pentachlorobiphenyl, respectively.

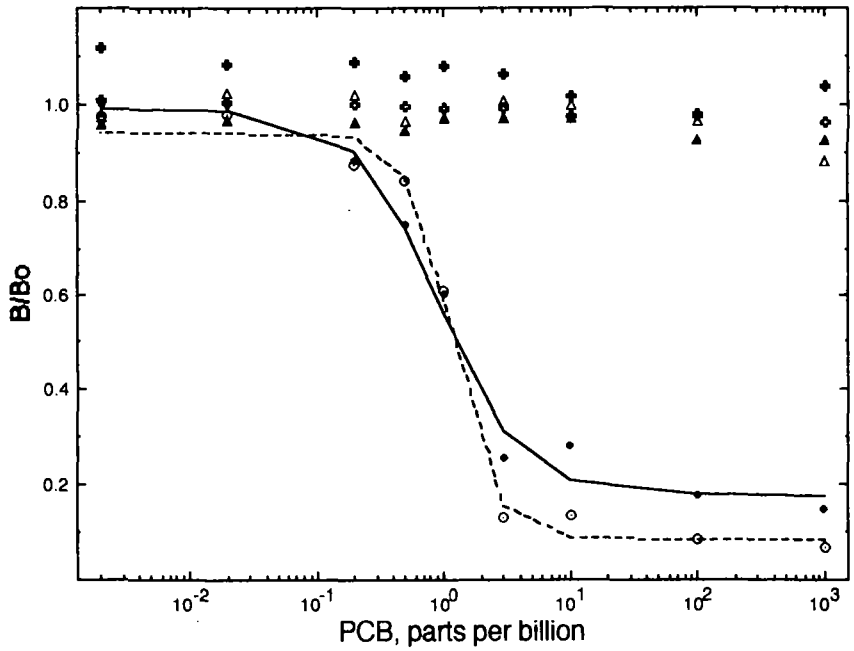

Table II. Cross-Reactivity in the EIA for PCB Congeners and Other Compounds.

IUPAC No.	Chlorination Pattern	Per Cent Cross-Reactivity Relative to PCB No. 126	
		EIA 5% Methanol	EIA 10% DMSO
Non-ortho Chlorinated PCB Congeners			
2	3 / -	<0.2	
12	34 / -	0.4	
13	3 / 4	<0.2	
14	35 / -	<0.2	
35	34 / 3	11	
37	34 / 4	0.4	
78	345 / 3	3.0	
81	345 / 4	<0.3	
77	34 / 34	96	192
126	345 / 34	100 (I_{50} 7 ppb)	100 (I_{50} 48 ppb)
169	345 / 345	<0.2	13
Ortho Chlorinated PCB Congeners			
8	2 / 4	<0.2	
28	24 / 4	<0.2	
52	25 / 25	<0.2	
70	25 / 34	0.4	
101	245 / 25	<0.2	
110	236 / 34	<0.2	
118	245 / 34	0.5	
153	245 / 245	<0.2	
Other Compounds			
3,3',4,4'-tetrabromobiphenyl			<0.4
3,3',4,4',5,5'-hexabromobiphenyl			<0.2
2,2',4,4',5,5'-hexabromobiphenyl			<0.2
2,3,7,8-tetrachlorodibenzo-p-dioxin			<1.0
2,3,7,8-tetrachlorodibenzofuran			<1.0
3,3',4,4'-tetrachlorodiphenyl ether			<1.0
3,4',5-trichloro-4-biphenylol		<0.2	
3,3',5,5'-tetrachloro-4,4'-biphenyldiol		<0.2	
p,p'-DDT		<0.2	
p,p'-DDD		<0.2	
1,2-dichlorobenzene		<0.2	
1,3-dichlorobenzene		<0.2	
1,4-dichlorobenzene		<0.2	
1,2,4-trichlorobenzene		<0.2	
3,4-dichloroaniline		<0.2	