Direct Immunoassay Detection of Selected Toxic, Coplanar Polychlorinated Biphenyl Congeners In Aroclors.

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INTRODUCTION

The most toxic PCB congeners are ortho unsubstituted and coplanar. They occur in much smaller amounts than the dominant, less toxic congeners in industrial PCB formulations and in environmental samples[1,2]. There is growing recognition that congener specific PCB analysis is required for an objective evaluation of risk and environmental impact[3,4]. The time, effort and expense associated with congener specific analysis by instrumental methods such as High Resolution Gas Chromatography (HRGC) places substantial constraints on the scope of risk assessment and site evaluation studies[5]. Immunoassay based analytical methods have demonstrated value for specific, high throughput screening as well as quantitative analyses of many environmental analytes[6]. We previously developed an Enzyme ImmunoAssay (EIA) which is specific for the most toxic, coplanar PCB congeners[7]: BZ#77, 3,3',4,4'-tetrachlorobiphenyl; BZ#126, 3,3',4,4',5-pentachlorobiphenyl; BZ#169, 3,3',4,4',5,5'-hexachlorobiphenyl. Although it was demonstrated that this assay could detect selected congeners in Aroclors, the non-linear Aroclor response of the assay prevented estimation of congener content. This presentation will discuss the development of procedures which solve the non-linearity problem and the application of these procedures to the analysis of selected congeners in Aroclors.

MATERIALS AND METHODS

The reported EIA was based on a specific monoclonal antibody (S2B1.9) and competitor conjugate (3,4-keto/HRP) combination[7]. These reagents have been formatted for use in a coated tube method[8]. The details of developing this method will be reported elsewhere. The standard assay procedure uses water as diluent. The sample, which is in methanol (10 ul), is added to the diluent (500 ul). After incubation for 15-1500 minutes to allow for analyte binding to the antibody, the tube is decanted and rinsed. Enzyme conjugate is added, incubated for 15 minutes and the tube decanted and rinsed. Chromogen is added, incubated for 30 minutes and the enzyme reaction stopped by the addition of 1N HCl. Sample OD is measured at 450 nm and the results evaluated by comparison to a negative control.

RESULTS

Assay Format and Cross-Reaction The assay, as initially developed[7], was not optimized for sensitivity or ease of manufacture. We have formatted the S2B1.9 monoclonal antibody and 3,4-keto/HRP enzyme conjugate into a tube based assay which can be manufactured in quantity and run in less than an hour once the incubation with analyte has been completed[8]. This optimized assay has an I_{85} (minimum detection limit) of 18 picograms and an I_{50} of 200 picograms for BZ#126. The specificity of this antibody and competitor combination where defined in the initial study[7] and have been confirmed in the new format[8]. As indicated in Table I, the assay is specific for the three most toxic, coplanar PCB congeners (BZ#77, 126, 169). The only cross-reactive non-target congener is BZ#35 (3,3',4-trichlorobiphenyl).

TABLE I. Cross-reaction Categories for Selected PCB Congeners.

100% Cross-reactive	BZ#126	3,3',4,4',5-pentachloro
5-20% Cross-reactive	BZ# 35	3,3',4-trichloro
	BZ# 77	3,3',4,4'-tetrachloro
	BZ#169	3,3',4,4',5,5'-hexachloro
0.2-2% Cross-reactive	BZ# 37	3,4,4'-trichloro
	BZ# 78	3,3',4,5-tetrachloro
	BZ# 81	3,4,4',5-tetrachloro (estimated)
	BZ#118	2,3',4,4',5-pentachloro
<0.01% Cross-reactive	BZ# 52	2,2',5,5'-tetrachloro
	BZ#153	2,2',4,4',5,5'-hexachloro

Method Development for Congener Specific Detection in Aroclors As described previously[7], the assay gave a non-linear congener response in the presence of Aroclors 1221-1260. We hypothesized that this problem was the result of interference by the vastly greater concentrations of common, but not cross-reactive, congeners. From an equilibrium perspective, the binding of BZ#126 is clearly favored over binding of a common congener like BZ#153 (2,2',4,4',5,5'-hexachlorobiphenyl) because BZ#153 is less than 0.01% cross-reactive to this antibody compared to BZ#126. This cross-reaction difference equals a difference in relative affinity[9] of at least 100,000. However, from a kinetic versus thermodynamic perspective, the system may be very slow in achieving equilibrium. An additional factor which may slow or even prevent equilibrium is the potential for the common, high concentration congeners to act as co-

solvents for the target congeners.

We evaluated the significance of the equilibrium and co-solvent factors by performing the assay using different concentrations of co-solvents and a range of assay incubation times. A preliminary experiment demonstrated that up to 8% methanol could be used as a co-solvent without significantly effecting assay sensitivity to BZ#126. Figure 1 illustrates the effect of increasing assay incubation time at a fixed methanol concentration of 4%. Clearly, the response of the assay to Aroclor 1260 is improved as the incubation time is increased. More importantly, the 1500 minute incubation assay curve for Aroclor 1260 is parallel to the BZ#126 standard curve as judged by comparison of the two curves at B/Bo 80, 50 and 20. Additional analyses provided confirmation that the modified assay is quantitatively responsive to selected congeners in the Aroclor mixture. In one experiment, a BZ#126 standard curve was prepared by varying the concentration of BZ#126 in the presence of a constant concentration (100 ng) of Aroclor 1260. Additionally, an Aroclor 1260 standard curve was prepared by varying the concentration of Aroclor 1260 in the presence of a constant concentration (100 pg) of BZ#126. In both cases, the standard curves were superimposed on the background value which was expected for the constant concentration analyte. Similar experiments with Aroclors 1242, 1248 and 1254 gave similar results.

Method Demonstration / Aroclor Analysis The target co-planar, toxic congeners occur in much smaller amounts than the less toxic congeners in the Aroclors[1,2]. Table II summarizes selected congener composition data for Aroclors 1221, 1242, 1248, 1254 and 1260. Based on these data and the immunoassay relationship between weight percent and percent cross-reaction, the immunoassay would be expected to detect about 0.1 weight percent of each Aroclor as specific congener equivalents when compared to the BZ#126 standard curve. Figure 2 gives the data obtained from the analysis of the five Aroclor formulations. The weight percent equivalents for the Aroclors, based on the BZ#126 standard curve, are: 1221 - 0.0018%, 1242 - 0.10%, 1248 - 0.18%, 1254 - 0.18%, 1260 - 0.048%. These values are in good agreement with expectation except for Aroclor 1221. The low value for Aroclor 1221 may simply reflect that this particular sample has a trace congener composition which is different from the sample data in Table II.

CONCLUSION

These results demonstrate that it is possible to use immunoassay for the analysis of specific congeners in Aroclors. These results are currently being extended to include the immunoanalysis of HRGC analyzed Aroclor samples. Additional studies will use S2B1.9 for the immunocapture and subsequent HRGC analysis of selected congeners from Aroclors.

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TABLE II. Composition of Aroclors 1221-1260[1,2]. Weight percent of selected congeners grouped by cross-reactivity per Table I.

	AROCLOR (Weight Percent)				
	1221	1242	1248	1254	1260
CONGENER					
100% Cross-reactive					
BZ#126 (3,3',4,4',5-)	--- (a)	---	---	---	---
5-20% Cross-reactive					
BZ# 35 (3,3',4-)		0.11		---	---
BZ# 77 (3,3',4,4'-)	0.40	0.50	0.30	---	---
BZ#169 (3,3',4,4',5,5'-)	---	---	---	0.08	0.05
0.2-2% Cross-reactive					
BZ# 37 (3,4,4'-)	0.05	0.30	0.32	---	---
BZ# 78 (3,3',4,5-)	---	---	---	---	---
BZ# 81 (3,4,4',5-)	---	---	---	---	---
BZ#118 (2,3',4,4',5-)	0.45	1.80	3.35	8.45	1.15
<0.01% Cross-reactive					
BZ# 52 (2,2',5,5'-)		4.04		5.18	0.56
BZ#153 (2,2',4,4',5,5'-)	0.15	0.75	0.93	4.73	13.50

(a) - less than 0.01%; BZ#78 and 81 are less than 0.05%; a blank indicates no data.

FIGURE 1. Comparison of the effect of sample incubation time on assay response. Plot of BZ#126 versus Aroclor 1260 standard curves. The BZ#126 standard curve used a 150 minute analyte incubation time. The three Aroclor 1260 standard curves are, from left to right, based on 1500, 150 and 15 minute analyte incubation times.

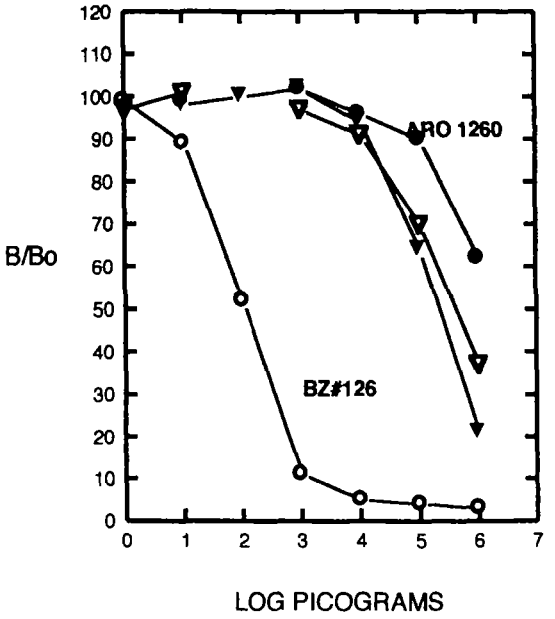

FIGURE 2. Congener specific analysis of Aroclors 1221 to 1260. The Aroclor plots, from left to right at B/Bo 50, are: 1248 / 1254, 1242, 1260, 1221.