Demonstration of a TEQ Selective PCB Immunoassay

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INTRODUCTION

PolyChlorinated Biphenyls (PCBs) are industrial products which were prepared as mixtures of the mono- to decachlorinated isomers (congeners) of biphenyl (1). PCBs are ubiquitous environmental contaminants (2). Of the 209 possible PCB congeners, only 14 have been assigned Toxicity Equivalence Factor (TEF) values on the basis of their "dioxin" equivalent toxicology (3). These 14, which are currently believed to be the most environmentally significant, are used in the calculation of a sample's Toxicity Equivalence (TEQ; 3). These congeners 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 initially developed an Enzyme ImmunoAssay (EIA) which was 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 (3). This assay was used in the development of procedures for the analysis of these selected congeners in complex PCB mixtures (6). Unfortunately, although this assay gave a result which is nominally correct on a congener concentration basis, it severely overestimates sample TEQ because the relative immunoassay cross-reactivity of PCB #77 versus PCB #126 (0.63 versus 1.00) does not parallel the relative TEF values of 0.0001 versus 0.1 for these two congeners (3,5). This presentation describes the development of a TEQ selective PCB immunoassay.

MATERIALS AND METHODS

The development of an appropriate antibody and assay competitor combination for this TEQ specific immunoassay was similar to our previous study (5) and will be described in detail elsewhere (6). Manufacture of these components into a coated tube EIA followed our usual method (7). Performance of the assay also followed our usual protocol (8).

RESULTS AND DISCUSSION

The primary design goals of a TEQ specific PCB immunoassay can be derived from PCB toxic congener occurrence in the environment. Two broad types of samples were considered: samples which have been directly contaminated with PCB containing wastes (e.g. hazardous waste site soils) and samples which have been indirectly contaminated with PCB's through food chain exposure (e.g. evaluation of the biotic environment and human food supplies). Congener specific data for several Aroclor samples (Aroclor 1242, 1254, 1260, data not shown (9)) and a limited set of biota samples (n = 7; copepod, clam, fish, bird, mammal, data not shown (10-13)) was used to determine the per cent TEQ contribution of the 14 PCB toxic congeners. The Aroclor data established that PCB # 118, 126, 156 and 170 are substantial contributors to TEQ with each congeners relative importance dependent on the particular Aroclor formulation (data not shown). The biota data indicated that the toxic congeners could be ranked based on mean percent occurrence (Table I) with PCB #126 accounting for nearly half

of the observed TEQ. These data established that PCB #126 is the primary analyte for TEQ determination.

The Aroclor and biota data could also be used to establish a sensitivity target for the assay. The biota data gave a TEQ concentration range of 10.0 - 104 pg TEQ per ug of total Aroclor (10-13). The Aroclor samples gave a TEQ concentration range of 3.7 - 19.8 pg TEQ per ug of total Aroclor (9). These samples also contained 3.0 - 27.1 pg PCB#126 per ug of Aroclor (Aroclor samples) and 52.8 - 667 pg PCB#126 per ug of Aroclor (biota samples). Based on these data and a typical sample size of 1 - 10 grams, the immunoassay must be sensitive to PCB#126 in the 10-100 pg per assay range to provide for both reasonable sample size and PCB#126 / TEQ sensitivity. PCB#126 sensitivity was evaluated in the EIA using multiple standard curves and both within day and between day timeframes. Combination of these results (n = 8) gave a minimum detection limit (mdl, operationally defined as 15% assay inhibition) of 5.2 pg (1.3 pg std dev), an assay midpoint (I50) of 50.6 pg (6.5 pg std dev) and an assay working range of 5 - 1,000 pg. These results demonstrate that this EIA is sufficiently sensitive for TEQ determination.

A TEQ specific assay must also be proportionately sensitive to the other PCB congeners which contribute to TEQ and insensitive to the non-TEQ congeners. Table I lists assay cross reaction for the 14 PCB congeners which are used in the determination of TEQ. This table demonstrates that there is reasonable correspondence between the TEF values assigned for these congeners and assay cross-reactivity, especially PCB #118 and 156's equivalence to PCB #126 and the 100 fold difference in cross-reactivity between PCB #77 and PCB #126. A more important test of the assay is based on the observation that the range of TEQ is typically 10-100 pg TEQ per ug of total sample Aroclor. Thus the non-TEQ or "common" congeners are present in 1,000 to 100,000 fold excess over the TEQ congeners. This observation indicates that the assay must be insensitive to the non-TEQ congeners. One approach to the evaluation of non-TEQ congener sensitivity is to measure the cross-reactivity of all 195 non-TEQ congeners. Clearly, this would require a major effort. Our approach was to develop a Model Aroclor 1254 which contains appropriate ratios of the major component non-TEQ congeners. This Model Aroclor 1254 was defined by combining congener specific data for 3 different lots of Aroclor 1254 (1,9,14) and calculating the mean occurrence of the approximately 80 congeners which are observed in these samples (data not shown). Further evaluation of this data established that the 24 congeners which are present at greater than 0.5 weight per cent concentration accounted for 68% of the total mass of these Aroclor 1254 samples. These 24 congeners where chosen as the Model Aroclor 1254 (TEQ free) sample set. Table I lists the cross-reactivity of the assay to these congeners. These data establish that the assay is relatively insensitive to these common congeners. More importantly, comparison of the calculated Model Aroclor 1254 assay response to the observed TEQ of a characterized Aroclor 1254 (Table II) indicates that the calculated assay response of 1.37 pg TEQ per ug Aroclor is small compared to the observed Aroclor 1254 TEQ of 19.8 pg TEQ per ug Aroclor. Thus the assay appears to be sufficiently selective to define the 10 - 100 pg TEQ per ug Aroclor levels which are expected to occur in the presence of the substantial excess of the Aroclor 1254 common congeners. This calculated result was confirmed experimentally. The Model Aroclor 1254 was prepared using the indicated weight per cent values (Table I) of the 24 non-TEQ congeners. Immunoassay of the Model Aroclor 1254 gave results (calculated 1.37 pg TEQ per ug Aroclor, observed 1.17 pg [0.32 std. dev.] TEQ per ug Aroclor; Table II) which are in excellent agreement with the calculated response.

The next step in the process of establishing assay performance was to evaluate Model Aroclor 1254 samples which had been spiked with various concentrations of PCB#126. These data, which were obtained by preparing PCB #126 standard curves in the presence of ug quantities of the Model Aroclor 1254, demonstrated that the assay could detect PCB #126 without substantial loss of sensitivity (PCB #126 I50 without Model Aroclor -> 56.6 pg versus I50 with 1-10 ug Model Aroclor -

> 58.8 - 78.8 pg). The final step in the development of this prototype was to evaluate the performance of the assay in the analysis of the characterized Aroclor 1254. The results in Table II [HRGC/HRMS calculated 19.8 pg TEQ per ug Aroclor, immunoassay calculated 14.6 pg TEQ per ug Aroclor, immunoassay observed 15.5 pg TEQ per ug Aroclor] clearly demonstrate excellent agreement between the calculated and observed immunoassay results and the HRGC/HRMS derived TEQ value.

In conclusion, we have successfully developed a prototype TEQ specific immunoassay. This demonstration is the first step in the process of assay validation using a broad range of real samples for the correlation of HRGC/HRMS versus immunoassay results.

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TABLE I. Cross-reaction and Related Data. Biota percent TEQ values not shown are less than 0.1%. Weight percent refers to the composition of the Model Aroclor 1254.

PCB Toxic Congeners

	PCB No.	Substitution	TEF	Cross-Reactivity	Biota TEQ %
Non-Ortho	77	3,4 / 3',4'	0.0001	0.0090	0.3
	81	3,4,5 / 4'	0.0001	0.0054	
	126	3,4,5 / 3',4'	0.1	* 1.00 *	43
	169	3,4,5 / 3',4',5'	0.01	2.32	3.6
Mono-Ortho	105	2,3,4 / 3',4'	0.0001	0.00017	5.1
	114	2,3,4,5 / 4'	0.0001	0.000063	
	118	2,4,5 / 3',4'	0.0005	0.000064	18
	123	3,4,5 / 2',4'	0.0001	0.0011	
	156	2,3,4,5 / 3',4'	0.0001	0.0043	21
	157	2,3,4 / 3',4',5'	0.0005	0.011	
	167	2,4,5 / 3',4',5'	0.00001	0.0093	
	189	2,3,4,5 / 3',4',5'	0.0001	0.092	
Di-Ortho	170	2,3,4,5 / 3',4',5'	0.0001	0.000083	7.5
	180	2,3,4,5 / 2',4',5'	0.00001	0.000023	1.8

Aroclor 1254 Common Congeners

	PCB No.	Substitution	Cross-Reactivity	Weight Percent
Tetrachloro-	44	2,3 / 2',5'	<0.000002	3.25
	49	2,4 / 2',5'	<0.000002	2.00
	52	2,5 / 2',5'	<0.000002	8.00
	66	2,4 / 3',4'	0.000058	1.50
	70	2,5 / 3',4'	0.00013	5.00
Pentachloro-	82	2,3,4 / 2',3'	0.000009	1.00
	84	2,3,6 / 2',3'	<0.000002	2.00
	85	2,3,4 / 2',4'	0.000005	1.50
	87	2,3,4 / 2',5'	0.000009	6.00
	92	2,3,5 / 2',5'	0.000005	1.50
	95	2,3,6 / 2',5'	0.000005	9.50
	97	2,4,5 / 2',3'	0.000005	2.50
	99	2,4,5 / 2',4'	<0.000002	4.75
	101	2,4,5 / 2',5'	<0.000002	13.00
	110	2,3,6 / 3',4'	0.000005	12.00
Hexachloro-	128	2,3,4 / 2',3',4'	0.000035	2.50
	132	2,3,4 / 2',3',6'	0.000005	2.00
	138	2,3,4 / 2',4',5'	<0.000002	8.25
	141	2,3,4,5 / 2',5'	0.000005	1.00
	149	2,3,6 / 2',4',5'	0.000018	5.00
	153	2,4,5 / 2',4',5'	0.000023	5.00
	158	2,3,4,6 / 3',4'	0.000005	1.25
	163	2,3,5,6 / 3',4'	0.000021	0.50
	168	2,4,6 / 3',4',5'	0.000028	1.00

Table II. Aroclor Immunoassay Results. Data compares calculated versus observed TEQ and PCB 126 equivalents results.

Model Aroclor 1254

Calculated	1.37 pg TEQ per uq Model Aroclor 1254
Immunoassay	13.7 pg PCB 126 Equivalents per ug Model Aroclor 1254
Observed	1.17 pg [n=3, 0.32 std dev] per ug Model Aroclor 1254
Immunoassay	11.7 pg [n=3, 3.2 std dev] PCB 126 Equivalents per ug Model Aroclor 1254

Wellington Aroclor 1254

Calculated TEQ	19.8 pg TEQ per ug Aroclor 1254
HRGC/HRMS	198 pg PCB 126 Equivalents per ug Aroclor 1254
Calculated	14.6 pg TEQ per ug Aroclor 1254
Immunoassay	146 pg PCB 126 Equivalents per ug Aroclor 1254
Observed	15.5 pg [n=3, 0.86 std dev] per ug Aroclor 1254
Immunoassay	155 pg [n=3, 8.6 std dev] PCB 126 Equivalents per ug Aroclor 1254