Fiber Optic Immunosensor for Polychlorinated Biphenyls

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A fiber optic immunosensor, consisting of a quartz fiber coated with partially purified polyclonal anti-PCB antibodies (Abs), was used to detect polychlorinated biphenyls (PCBs). The optical signal was generated by the binding and subsequent fluorescence of the fluorescein (FL) conjugate of 2,4,5-trichlorophenoxybutyrate (TCPB) to the Ab-coated fiber. The Abs, immobilized on the fiber, bound TCPB–FL with high affinity, saturably and reversibly. TCPB and PCBs competed with TCPB–FL for binding and decreased fluorescence in a concentration-dependent manner, with a detection limit of 10 ppb TCPB. The biosensor detected 1 ppm Aroclors, but not polychlorinated pesticides, polychlorinated phenols, or trichlorobenzene. It was more selective for TCPB at 1 ppm than the four tested Aroclors (i.e. 1016, 1232, 1250, and 1262) by 1.4-, 2.2-, 5.5-, and 3-fold, respectively. It detected Aroclor 1242 added to river waters and standard reference soil samples with reasonable accuracy. Advantages of this biosensor are its fast detection time (seconds to minutes), high sensitivity, and reversibility.

Keywords: *Polychlorinated biphenyls, detection; biosensors; immune detection; polychlorinated hydrocarbons, insecticides*

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a highly stable family of compounds with paired phenyl rings and various degrees of chlorination. They were used mostly as insulators in electrical equipment and were banned from use over 12 years ago. Yet, they persist in the environment (Erickson, 1986) and have global environmental distribution. They are detected in water, food, human tissues (Jensen, 1989), milk (Mullin et al., 1981; Johansen et al., 1994), and urine (Kutz et al., 1992) and in high concentrations at Superfund sites in the United States. Occupational exposure to high doses of PCBs can cause dermal toxicity (James et al., 1993), as well as malignant melanomas and brain cancer (Rogan and Gladen, 1992; Sinks et al., 1992). They have also been found to alter growth and development in humans (Lambert and Hsu, 1992). Exposure of chicken embryos to as little as 0.01 ppm PCBs for 15 days resulted in retardation of growth and increased frequency of malformations (Hatano and Hatano, 1994). Although there are 209 possible PCB congeners, only a few account for most PCBs detected in fish, birds, mammals, and invertebrates (McFarland and Clarke, 1989), and even fewer are potentially toxic. However, there are reports that individuals who consume relatively large amounts of fish may have elevated serum PCB levels that may be hazardous (Baker et al., 1980; Kreiss et al., 1981).

Since commercial PCBs released in the environment are usually mixtures, their detection and analysis is usually a complicated process. Analysis of commercial PCB mixtures or PCBs in milk and blood has been accomplished by gas chromatography (Safe et al., 1985), radioimmunoassay (Albro et al., 1979; Newsome and Shields, 1981; Johanson et al., 1994), and enzyme-linked immunoassay (ELISA) (Johnson et al., 1994). Immunoassay methods were reported to be comparable to GC- and HPLC-based methods for detection of total PCBs.

Recent advances in optical technology as well as the availability of a wide range of immunochemicals have led to the development of biosensors that are cost-effective, user friendly, sensitive, and field portable. Biosensors are analytical devices that utilize the specificity of the binding site of a receptor, enzyme, antibody (Ab), or DNA to detect an analyte by coupling these proteins to transduction elements (e.g. glass electrodes, silicon chips, or optic fibers). Biosensors have certain advantages that may improve on ELISA. These advantages include speed (with detection in seconds to minutes), reusability for multiple assays, simplicity, and potentially low cost per assay. During the past 6 years, we have used biosensors (fiber optic evanescent fluorosensors and potentiometric biosensors) to study drug– and toxin–receptor interactions and their specificities (Rogers et al., 1989, 1991a, 1992; Eldefrawi et al., 1992) and to detect and quantitate cocaine (Devine et al., 1995) and anticholinesterases (Rogers et al., 1991b) including insecticides (Anis et al., 1992; Fernando et al., 1993). The more potent of these enzyme inhibitors (e.g. paraoxon) could be detected at low concentrations (i.e., 1.5×10^{-7} M), while higher concentrations were required to detect low-potency inhibitors (such as parathion) (such as 10^{-3} M).

While enzyme-based (i.e. acetylcholinesterase) and receptor-based (i.e., nicotinic) biosensors are generic in their detection capabilities of substrates and inhibitors or agonists and antagonists, respectively, Ab-based biosensors can be highly selective. We utilized Abs directed toward a bovine serum albumin–parathion conjugate as sensing elements to construct a fiber optic

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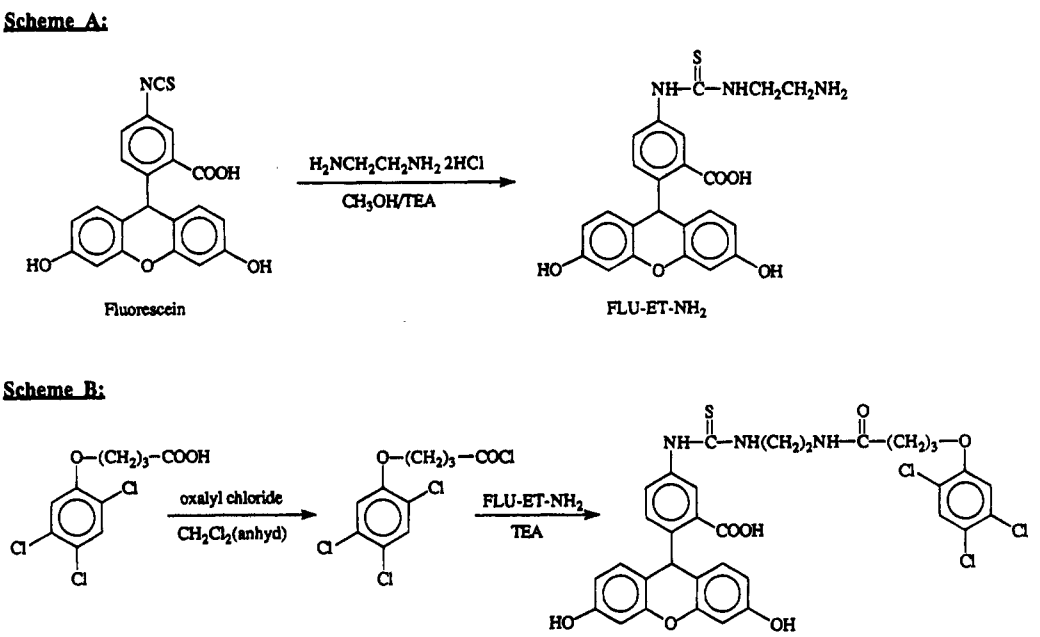

Figure 1. Synthetic scheme for the 2,4,5-trichlorophenoxybutyric acid–fluorescein conjugate (TCPB–FL). Scheme A is for synthesis of the fluorescein ethylenediamine derivative. Scheme B is for synthesis of TCPB–FL from the derivative.

biosensor (i.e. immunosensor) to detect this pesticide (Anis et al., 1992). The biosensor specifically detected 0.3 ppb of parathion and was 100-fold more selective for parathion than for its more potent oxidative metabolite paraoxon (Anis et al., 1992).

Immunosensors have also been developed to detect herbicides such as atrazine in surface water and soil extracts (Oroszlan et al., 1993), as well as imazethapyr and other imidazolinone herbicides in soil samples (Anis et al., 1993; Wong et al., 1993). For the imazethapyr biosensor, a fluoresceinated imazethapyr probe was used as the optical signal generator, which bound to the Abs immobilized on the fiber. The probe that bound to the immobilized Ab was competitively and reversibly displaced by imazethapyr. This biosensor was regenerable for multiple use because of the reversibility of binding of the antigen to the Ab. It was selective for three imidazolinone herbicides (imazapyr, imazaquin, and imazmethabenz methyl) studied but had at least 100-fold lower affinity for non-imidazolinone herbicides. Matrix material in soil extracts had minimal effect on this biosensor.

In the present study, a protocol similar to the one used for the imazethapyr biosensor was utilized to develop a sensitive and reversible biosensor for detection of PCBs found in Aroclors. The sensitivity and selectivity of this biosensor to various Aroclors, polychlorinated benzene, phenol, and insecticides are reported.

MATERIALS AND METHODS

Chemicals. Polychlorinated benzene and phenols (reagent grades, purity 98–99%) were obtained from Aldrich Chemicals (Milwaukee, WI), *p,p'*-DDT and chlordane were from Chem-Service (West Chester, PA), and human [^{125}I] IgG, 0.5 μCi/μg, was from NEN DuPont (Boston, MA). 2,4,5-Trichlorophenoxybutyric acid (TCPB) and 2,4-dichlorophenoxyacetic acid (2,4-D, reagent grade, purity 99%) as well as all other chemicals (reagent grade) were from Sigma Chemical Co. (St. Louis, MO). 2,4,5-Trichlorobutyrate–fluorescein conjugate (TCPB–FL) was synthesized from fluorescein isothiocyanate and TCPB as shown in Figure 1.

Instrumentation. A portable fluorometer, designed and built by ORD Inc. (North Salem, NH) [Block and Hirschfeld, 1986; see Rogers et al. (1989)] was used to measure the fluorescence generated on the surface of 1 × 60 mm quartz fibers by the fluorescent ligands within the evanescent zone. Excitation (485/20 nm) and emission (530/30 nm) band-pass filters were used in conjunction with the fluorescein probe. The fibers were mounted vertically in a flow cell of 46 μL capacity, which was exchanged every 14 s. Prior to use, the quartz fibers were washed in hot ethanol (70 °C) for 30 min and stored in anhydrous ethanol. Prior to protein immobilization, the fibers were dried in N_2 atmosphere.

Purification of Anti-PCB γ-Globulins. The Abs used as sensing elements were provided by Dr. Jeanette Van Emon at the U.S. EPA Environmental Monitoring Systems Laboratory at Las Vegas. The antiserum was raised against an alkoxy derivative of 4-hydroxy (2,2',4',5,5'-pentachloro-4-biphenylol) conjugated to keyhole limpet hemocyanin (Johnson and Van Emon, 1994). The polyclonal Abs were isolated from the rabbit antiserum by passage through a protein A chromatography column (Acti-Disk; FMC) following the manufacturer's recommendation. Briefly, 1 mL of immune serum mixed with 4 mL of phosphate-buffered saline (10 mM disodium phosphate, 154 mM NaCl, pH 7.2) (PBS) was loaded on the column, followed by 20 mL of PBS at a 2 mL/min flow rate. The IgG fraction was then eluted with 0.1 M glycine-hydrochloride, pH 2.8, at a flow rate of 4 mL/min, and 1.5 mL fractions collected. Protein-containing fractions (determined by absorbance at 280 nm) were pooled and dialyzed extensively against PBS. The protein content of the pooled fractions was determined according to the Lowry et al. (1951) method and stored at 4 °C in the presence of 0.02% sodium azide until use.

Immobilization of Antibodies on the Quartz Fibers. The polyclonal Abs were adsorbed noncovalently on the surface of the quartz fibers by immersing the fibers in PBS containing the IgG fraction at 50 μg/mL either for 16 h at 4 °C or for 2 h at 23 °C. No attempt was made to determine the amount of the anti-PCB-Abs adsorbed onto the fibers. However, data presented under Results will show that the amplitude of the optical signal was directly related to the concentration of Ab used in the immobilization procedure. Furthermore, using [^{125}I]IgG as a tracer (using the 2 h, 23 °C incubation) resulted in an average of 0.12 μg of IgG adsorbed per fiber.

Fluorescence Measurement. The antibody-coated fibers were mounted in the flow cell and perfused with PBS containing 0.1% casein, which was added to the buffer to reduce nonspecific binding of fluorescein conjugate to the fibers. The baseline of light energy reaching the detector was recorded on a strip chart recorder. A 10 min baseline record was obtained before addition of TCPB–FL to the flow buffer. At

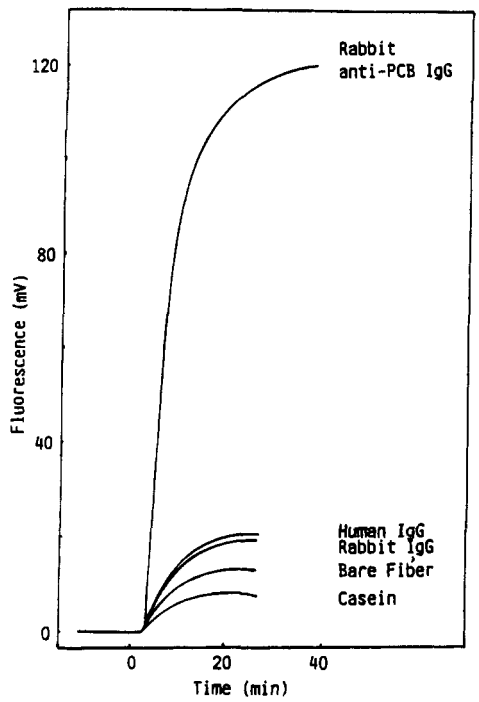

Figure 2. Specificity of the fluorescence signal. The fluorescence transmitted by TCPB–FL, bound to the fiber coated with the rabbit anti-PCB IgGs, compared to that of the casein-coated fiber, the bare fiber, and those coated with human IgG and rabbit IgG.

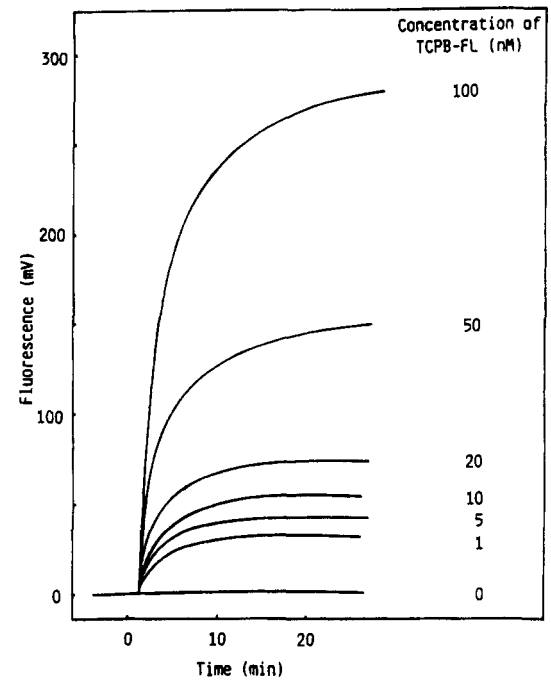

Figure 3. Dependence of the rate of fluorescence increase and the steady state fluorescence level on the concentration of TCPB–FL in the perfusing buffer. Quartz fibers were incubated in 50 μg/mL anti-PCB rabbit IgG in PBS (10 mM, pH 7.4) solution overnight at 4 °C. The same fiber was used to generate all curves. The fiber was perfused with PBS after each TCPB–FL concentration until fluorescence returned to baseline level.

a flow rate of 0.2 mL/min it took 14 s to exchange the buffer inside the flow cell completely. The fluorescence, generated in the evanescent zone from binding of TCPB–FL to the Ab on the surface of the fiber, was transmitted through the quartz fiber to the detector, translated into voltage, and recorded. Fluorescence transmitted through the fiber was directly correlated to the occupancy of Abs by 2,4,5-TCPB–FL. Thus, the optical signal expresses the binding reaction as it occurs.

Detection of Polychlorinated Hydrocarbons. Polychlorinated hydrocarbons (PCHs) that were recognized by the Abs competed with TCPB–FL for binding to the Abs, thereby reducing fluorescence. Unless otherwise indicated, Ab-coated fibers were perfused with PBS containing 25 nM 2,4,5-TCPB–FL until the fluorescence reached a steady state (usually <20 min). The PCH, at the desired concentration, was then added to the flow buffer, and the changes in fluorescence occurring within the next 5 min were recorded. The PCH was then removed from the flow buffer, the fluorescence was allowed to return to steady state, and another PCH was tested.

RESULTS

Specificity of the Fluorescent Signal. The measured fluorescence was generated by TCPB–FL within the evanescent zone. As expected, bare fibers (i.e. quartz fibers that have no proteins on their surface) bound relatively little TCPB–FL, resulting in a small optical signal (Figure 2). This nonspecific binding was reduced by half when the quartz fiber was coated with casein (1 mg of casein/mL in PBS) prior to use. This result suggested that casein masked some of the sites on the surface of the fiber which bound TCPB–FL. The fiber coated with the rabbit anti-PCB Abs generated significant fluorescence above background, due to the accumulation (i.e. specific binding) of TCPB–FL within the evanescent zone (Figure 2). Fibers, coated with rabbit or human IgGs, bound ≈15% of the TCPB–FL bound by the specific Abs. Thus, nonspecific binding of TCPB–FL ranged between 7% and 15% for pretreatment with casein and nonspecific IgG, respectively. The value of 15% was used to correct for nonspecific binding in subsequent experiments using the same concentration of TCPB–FL (i.e. 25 nM).

Effect of Concentration of TCPB–FL on the Fluorescence Signal. Since the fluorescence signal was generated by TCPB–FL, which bound specifically to immobilized anti-PCB Abs, it was expected that as the concentration of the TCPB–FL increased, the fibers carrying a discrete number of Abs would accumulate the fluorescent probe. The fluorescence transmitted by the Ab-coated fibers should then increase in a concentration-dependent manner and exhibit saturation kinetics. When Ab-coated fibers were perfused with different concentrations of TCPB–FL, varying from 1 to 100 nM, the amplitude of the fluorescence was indeed concentration-dependent (Figure 3). The time to reach steady state fluorescence also increased with higher TCPB–FL concentrations. The change in fluorescence, measured during the initial 2 min, was used to determine the initial rate of fluorescence increase.

When a single fiber was perfused with incrementally increasing concentrations of TCPB–FL, the optical signal increased stepwise and established steady state fluorescence levels similar to those recorded from individual fibers (data not shown). The steady state fluorescence and the initial rate of fluorescence increase were linear with increasing concentrations of TCPB–FL up to concentrations of 50 nM. At higher concentrations, the fluorescence appeared to saturate (Figure 4). Accordingly, 25 nM TCPB–FL was selected as the fluorescent probe concentration because it gave a fairly strong optical signal.

Fluorescence Signal Effects Due to Antibody Immobilization Conditions. Since the observed fluorescence signal reflected the binding of TCPB–FL to Abs immobilized on the surface of the fiber, it was

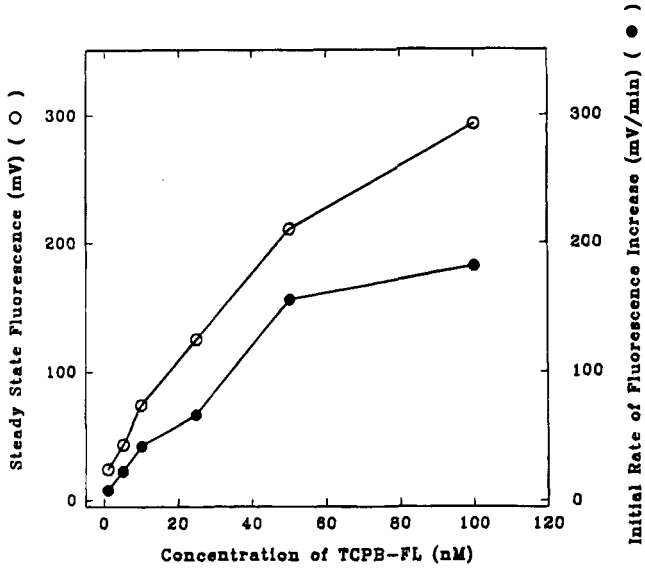

Figure 4. Effect of increasing concentrations of TCPB in the flow buffer on the steady state fluorescence and the initial rate of fluorescence increase. Fibers were coated with Abs as described in the legend of Figure 3.

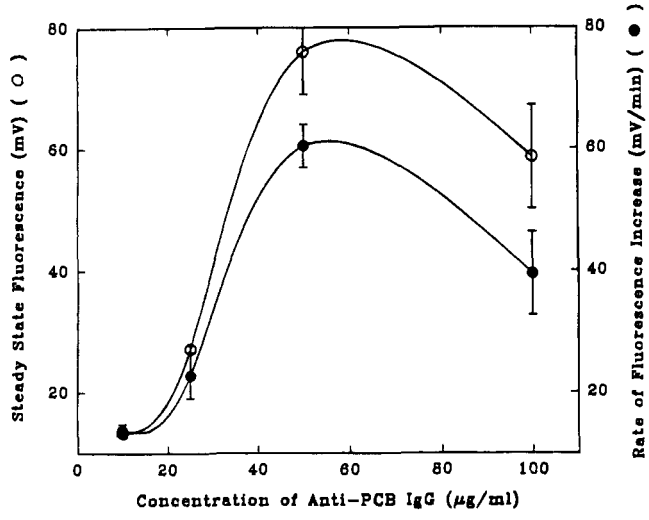

Figure 5. Effect of increasing concentrations of anti-PCB rabbit IgG in PBS during immobilization (4 °C overnight) on the amplitudes of steady state and rate of fluorescence upon perfusion with TCPB–FL (25 nM). Fibers were incubated at 4 °C overnight. Symbols and bars represent means of three measurements on triplicate fibers ± standard deviations.

expected that the concentrations of Abs on the surface (i.e. density of the binding sites) would affect the optical signal. The concentrations of anti-PCB Abs, in the incubation mixture for immobilization, were varied from 10 to 100 μg/mL. Three fibers were incubated in 0.3 mL of PBS containing 10, 25, 50, or 100 mg IgG/mL of PBS for 16 h at 4 °C. After the fibers were mounted in the flow cell, the fluorescence, generated by adding 25 nM TCPB–FL to the flow buffer, was recorded and the initial rate of, as well as the steady state of, fluorescence was plotted against the Ab concentration (Figure 5). The fluorescence response increased with increasing concentrations of Abs (up to 50 μg/mL) used during immobilization then decreased at 100 μg/mL. The observed 10–20% SD reflects variability that is likely to be associated with differences in the densities of adsorbed Abs on the fiber. Since the concentration of 50 μg/mL gave the highest optical signal, it was selected for use in all subsequent experiments.

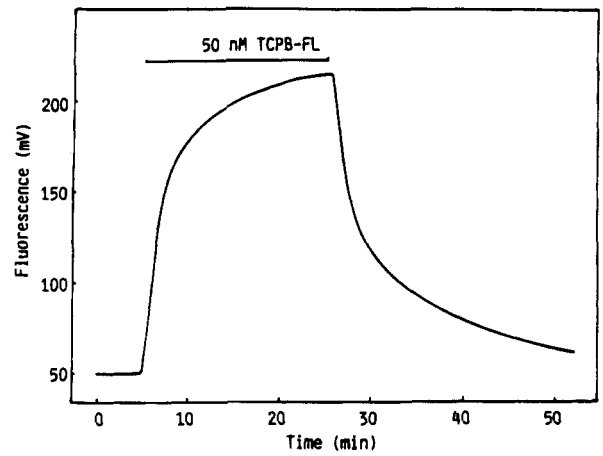

Figure 6. Time course of association of TCPB–FL to and its dissociation from the Ab-coated fibers. Bar indicates the time that TCPB–FL (50 nM) was present in the PBS flow buffer.

Preliminary experiments indicated that fibers incubated in 50 μg/mL IgG at 23 °C for 2.5 h bound more TCPB–FL and transmitted more fluorescence than fibers incubated at 4 °C for 16 h (data not shown). Although a fairly short incubation time (i.e. <60 min) at 23 °C was adequate to generate a fluorescent signal (data not shown), 150 min at 23 °C was selected as the standard immobilization condition to optimize signal and for convenience.

Reversibility of Binding of TCPB–FL to the Ab-Coated Fiber. Binding of TCPB–FL to the Ab-coated fiber was reversible (Figure 6). Upon the introduction of 50 nM TCPB–FL into the flow buffer, the fluorescence increased, thereby reporting binding of TCPB–FL to the Ab, and approached equilibrium (i.e. steady state). When TCPB–FL was removed from the flow buffer after 20 min, the fluorescence decreased as the bound TCPB–FL dissociated from the fiber. Although dissociation was initially rapid, after 60 min, the fluorescence level was still higher than the initial baseline level. A plot of the logarithm of fluorescence vs time indicated a fast dissociating component (43.2%) that had $T_{1/2}$ of 3 min and a slower dissociating component (56.8%) that had $T_{1/2}$ of 7.8 min. Thus, TCPB–FL bound to at least two populations of binding sites on the Ab-coated fiber. The dissociation rate constants k_{diss} or k_{-1} of the fast and slow components were calculated from the formula $k_{-1} = 0.693/T_{1/2}$ and found to be 0.231 and 0.088 min^{-1}, respectively. An apparent rate constant of association (k_{app}) of 2.29 min^{-1} was calculated from the linear plot of Ln FL_{eq}/(Ln FL_{eq} − Ln FL_t) vs time (Limbird, 1987). The steady state fluorescence was used to represent FL_{eq}. The association rate constant k_1 was then calculated from the equation $k_1 = k_1'/[L]$ since $k_{app} = k_1' + k_{-1}$ and two k_{-1} values were obtained. Both k_{-1} values were used to obtain k_1 values of 4.4×10^7 and 4.0×10^7 M^{-1}. The average of the two values (4.2×10^7 M^{-1}) was used to calculate the equilibrium dissociation constant K_D values. The K_D for the fast component was 1.04×10^{-8} M and for the slow component 4×10^{-9} M.

Concentration-Dependent Displacement of TCPB–FL by TCPB. When TCPB was added to the flow buffer containing TCPB–FL after steady-state fluorescence was achieved, it displaced the bound TCPB–FL and reduced fluorescence in a concentration-dependent manner (Figure 7). The rate of decrease in fluorescence, calculated from the slope of the initial 2 min segment of the curve following addition of TCPB

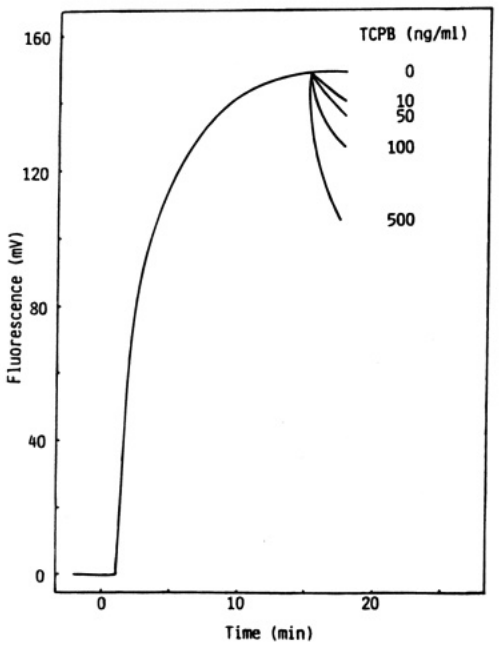

Figure 7. Concentration-dependent displacement of bound TCPB–FL from the Ab-coated fiber by different concentrations of TCPB. This experiment was done on a single fiber. The fiber was incubated with 50 μg/mL Ab at 4 °C overnight; then the lowest concentration of TCPB was introduced after 15 min of perfusion with PBS containing 50 nM TCPB–FL to establish a steady state. The initial rate of fluorescence decrease (initial 2 min) was recorded. The fiber was then perfused with PBS containing TCPB–FL for 10–20 min to reestablish the steady state before introduction of the next TCPB concentration.

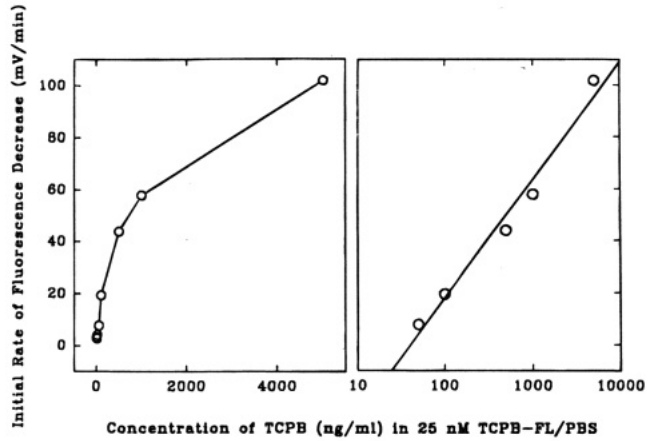

Figure 8. Reduction in fluorescence caused by displacement of TCPB–FL with increasing concentrations of TCPB: (left) initial rate of decrease; (right) Linear regression of the data. Symbols are means of triplicates.

to the flow buffer, was greater with increasing TCPB concentration. The displacement of the fluorescent probe by increasing concentrations of TCPB was saturable, and a logarithmic transformation yielded a linear response (Figure 8). A single fiber was used to make all measurements, which was feasible because of the reversibility of TCPB binding. After each measurement, the fiber was perfused with the flow buffer containing TCPB–FL until the fluorescence returned to steady state level. The time required increased as the concentration of TCPB increased. The rate 5000 ng/mL TCPB was selected to represent 100% displacement. Linear transformation of the data (Figure 8) was used to generate a standard curve for comparison of other PCHs. The IC_{50} value for TCPB of 750 ng/mL is equal to 2.64 μM. Using the Cheng–Prusoff equation [(K_i = $IC_{50}/(1 + [L]/K_D)$] (Cheng and Prusoff, 1973), the equilibrium constant of inhibition of TCPB–FL binding by TCPB (K_i) was calculated to be 7.8×10^{-9} M.

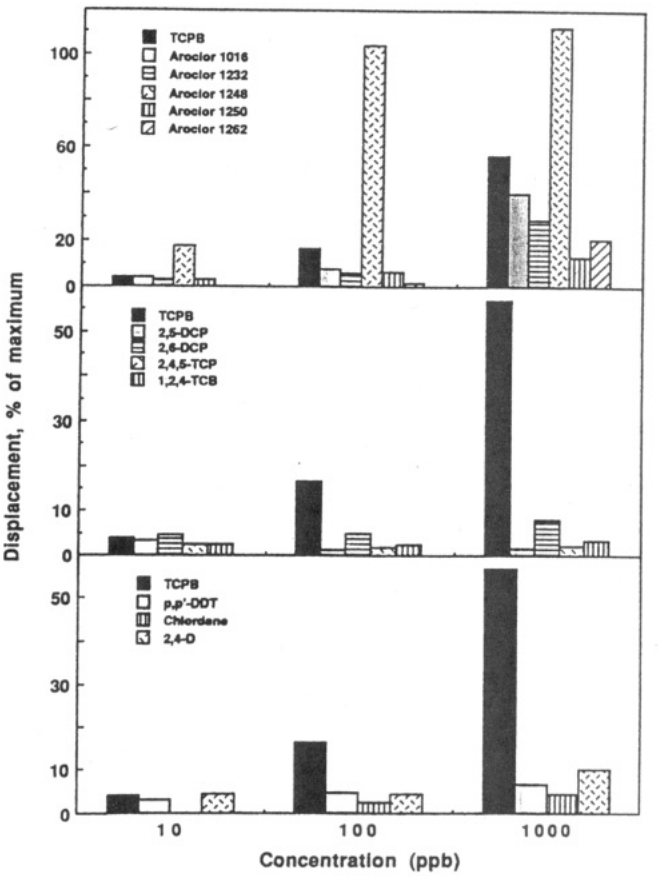

Figure 9. Selectivity of the biosensor as shown by the displacement of bound TCPB–FL by PCBs added to the flow buffer at 10, 100, and 1000 ppb: (A, top) PCB mixtures (Arochlors); (B, middle) polychlorinated di- and trichlorophenol or benzene; (C, bottom) polychlorinated pesticides.

Cross-Reactivity with PCHs. Because antiserum toward a specific PCB congener was used to construct this immunosensor, it is expected that these Abs would cross-react with other PCBs. Five PCB mixtures (i.e. Aroclors) were tested and showed cross-reactivities ranging from 23.1 to 197% of the relative response to TCPB (each at 1000 ng/mL) (Figure 10 and Table 1). The biosensor was more selective for TCPB at 1 ppm than the four Aroclors: (1016, 1232, 1250, and 1262) by 1.4-, 2.2-, 5.5-, and 3-fold, respectively. However, it was 5.8-fold more sensitive in detecting 10 ppb Aroclor 1248 than TCPB (Figure 9). In the concentration range from 1 to 1000 ppb, Aroclor 1248 showed a concentration-dependent displacement of the bound TCPB–FL from the anti-PCB Ab-coated optic fiber (Figure 10).

Aroclor 1248 is a mixture of PCBs. An average molecular mass of 290 Da based on pentacholorobiphenyl was used to calculate the inhibitory equilibrium constant as a measure of affinity of the biosensor for this PCB mixture. The average molecular mass was used to convert the IC_{50} value (Figure 10) into molar concentration, and the Cheng–Prusoff equation [K_i = $IC_{50}/(1 + [L]/K_D)$] was used to calculate a K_i of 1×10^{-8} M. In other words, the immobilized Abs seem to have similar affinities for TCPB–FL and Aroclor 1248 despite the high cross-reactivity of this PCB mixture with the sensor (Table 1).

On the other hand, the immunosensor had low cross-reactivity with a number of other PCHs. Dichlorophe-

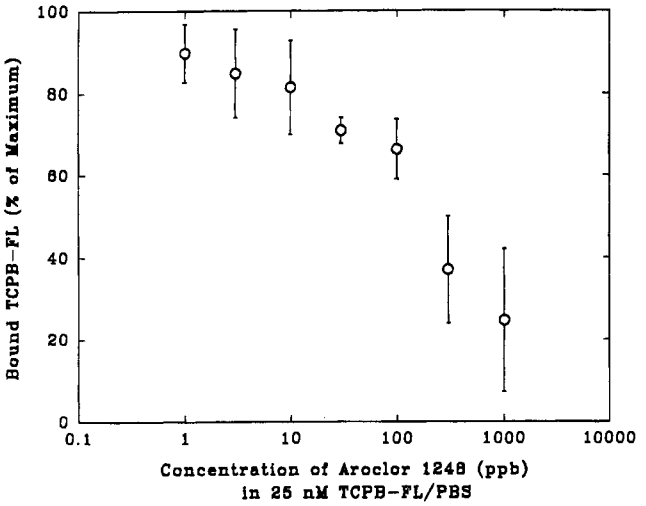

Figure 10. Concentration dependence of displacing bound TCPB–FL with Aroclor 1248. Percent displacement was obtained by dividing the slope of fluorescence decrease during the initial 2 min following the addition of Aroclor 1248 to the flow buffer (at the indicated concentrations) by the slope of fluorescence decrease following addition of 5 ppm TCPB (which gives maximal displacement). Symbols represent means of triplicate measurements, each on a separate fiber, and the bars represent standard deviations.

Table 1. Cross-Reactivities of PCBs and Other Polychlorinated Hydrocarbons

compound	cross-reactivity index[a]
Aroclors	
1016	70.6
1232	50.7
1248	197
1250	23.1
1262	35.9
polychlorophenol/benzene	
2,5-DCP	2.8
2,6-DCP	5.4
2,4,5-TCP	3.6
1,2,4-TCB	6.2
pesticides	
p,p'-DDT	11.6
chlordane	8.1
2,4-D	17.6

[a] Cross-reactivity values of PCBs and other polychlorinated hydrocarbons are calculated as follows: The initial rate of fluorescence decrease caused by the addition of 1000 ng/mL of TCPB to the flow buffer after fluorescence reached a steady state is considered as 100. The initial rate of fluorescence decrease in the presence of 1000 ng/mL of each compound is divided by that for TCPB to obtain the cross-reactivity index.

nols (2,5-DCP and 2,6-DCP), trichlophenol (2,4,5-TCP), and trichlorobenzene (1,2,4-TCB) were hardly detected at 1 ppm (Figure 9 and Table 1). The immunosensor showed slightly higher cross-reactivity with the insecticides DDT and chlordane and even more for the herbicide 2,4-D (Figure 10), which is similar in structure to 2,4,5-TCPB.

Detection of Aroclor 1242 in Two Surface Water Samples and Soil Extracts. A displacement curve of TCPB–FL binding to the fiber optic immunosensor with increasing concentrations of Aroclor 1242 was generated, and a least squares regression of the displacement curve (Figure 11) yielded a regression coefficient of 0.97. This was used as a calibration curve for Aroclor 1242 added to two water samples obtained from the Potomac and Chesapeake Bay and soil extracts. Recoveries of Aroclor 1242 added at low concentrations (10 and 100 ng/mL) to the waters and assayed the same day or at

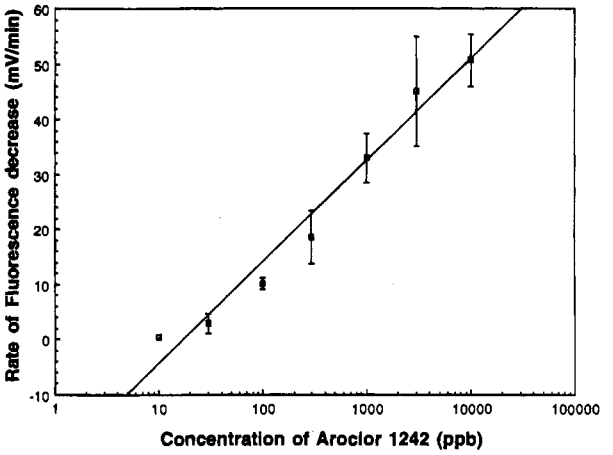

Figure 11. Linear transformation of the concentration-dependent displacement of TCPB–FL with Aroclor 1242.

Table 2. Recovery of Aroclor 1242 from River Water Using the Fiber Optic Immunosensor[a]

added Aroclor 1242 (ng/mL)	measured Aroclor 1242 (ng/mL)	SD	% recovery
10	8.91	1.38	89.26
100	70.81	22.58	70.96
500	547.54	180.15	109.51
1500	1707.37	315.21	113.80

[a] Each sample was assayed in triplicate. Measured Aroclor 1242 (ng/mL) respresents the mean of the three assays.

Table 3. Recovery of Aroclor 1242 from Methanol Extracts of Standard Reference Material (SRM) Soils

extraction[a] and analysis	amount of Aroclor 1242 added to SRM (mg/kg)					
	0.5	1.5	8.0	25	45	100
K1	2.10	3.06	3.51	25.7	29.7	97.4
2	1.93	5.00	9.64	44.7	42.8	154
3	2.19	4.16	5.89	31.3	42.7	70.5
expected	0.50	1.50	8.00	25.0	45.0	100
mean[b]	2.12	4.07	6.35	33.9	38.4	119
% error	324	171	21	36	15	19

[a] The numbers 1–3 refer to three fortified soil standards for each concentration. [b] Mean of the values for samples 1–3.

high concentrations (500 and 1500 ng/mL) and assayed after 7 days ranged from 71 to 101% for the low concentrations and from 109 to 114% for the high concentrations (Table 2). Aroclor 1242 was extracted from standard reference material (SRM) soils using a simple and rapid methanol extraction procedure. Mean values were determined from three or four extracts (Table 3). Percent error values were greatest at the lower concentrations when the biosensor overestimated the concentration of Aroclor 1242. The relative standard deviation values averaged about 30%.

DISCUSSION

Although TCPB–FL was observed to bind to the quartz fibers in the absence of Ab or to fibers coated with casein or immunoglobulins, binding of TCPB–FL to anti-PCB Abs, which were immobilized on the quartz fibers, gave a significantly greater response (Figure 2). In previous studies, nonspecific binding was discovered to be a major problem in determining the specific binding of FL–α-bungarotoxin to nicotinic receptor protein-coated fibers (Rogers et al., 1989). However, pretreatment of quartz fibers with PBS containing 0.1 mg/mL bovine serum albumin, and the use of low concentrations of FL–α-bungarotoxin, eliminated the

problem. Although using the same strategy in the current study did not totally eliminate nonspecific binding, the data were corrected for the presence of nonspecific binding, as is commonly practiced in radioactive ligand binding assays (Limbird, 1987).

The observed fluorescence resulting from the accumulation of probe in the evanescent zone using this system has been used to accurately measure bimolecular binding (Rogers et al., 1991). In the present study, the optical signal (i.e. fluorescence) increased with increasing concentration of TCPB–FL, due to a higher occupancy level for immobilized binding sites. The time course of the reaction (Figure 3) indicates that the binding reaction reaches steady state fairly quickly (<20 min), especially at low TCPB–FL concentrations. Since the fluorescence transmitted through the fiber reports the binding event, it is to be expected that higher concentrations of TCPB–FL would yield higher fluorescence (Figures 3 and 4). According to the law of mass action, the steady state fluorescence resulting from the event correlates directly with the concentration of the reacting species, i.e. the Abs and TCPB–FL (Figure 5). Therefore, increased concentrations of either of these reactants would produce higher fluorescence. Furthermore, the reaction should show saturation behavior since the fiber carries a limited number of binding sites. Variability in density of the immobilized Abs is expected to contribute to variable levels of the optical signal. Variability due to this source can be dramatically reduced by using each fiber as its own control.

The affinity of an Ab for its antigen is a basic experimental parameter in the thermodynamic approach to the molecular basis of their interactions (Goldberg and Djavadi-Ohaniance, 1993). If the analyte binds reversibly with low affinity to the Ab, the biosensor is easily regenerated for multiple measurements; if it binds with very high affinity, its regeneration is much slower. The determination of the affinity value is complicated not only by the heterogeneity of Abs but also by the multivalence of Abs and their immobilization on the glass fiber. This is affected by how the Ab is positioned on the fiber and any steric hindrance resulting from the density of immobilized Abs. The apparent affinity of this biosensor for TCPB–FL ($K_D = 0.4, 1.0 \times 10^{-8}$ M) results in fast dissociation of the bound TCPB–FL (Figure 6) and its competitive displacement by unlabeled TCPB (Figures 7 and 8) or other chemicals (Figures 9–11) that cross-react with the Abs (Table 1). This is advantageous because it allows for the rapid regeneration of the biosensor and return of steady state fluorescence upon fiber perfusion with analyte-free flow buffer.

The 5.8-fold higher selectivity of this biosensor for Aroclor 1248 than for TCPB (Figure 9 and Table 1) is not surprising in view of the fact that the immunogen for the antiserum was a PCB congener present in this Aroclor. Nevertheless, these Abs recognize and bind TCPB with fairly high affinities. Although the affinity of the biosensor for Aroclor 1248 is higher than for TCPB, they both have K_i values close to 10^{-8} M. This paradox may be caused by using an average molecular mass value for a pentacholorobiphenyl to calculate the K_i value. Use of a single PCB as the analyte would eliminate this problem. Nevertheless, the biosensor showed a high specificity for certain of the Aroclors, with low cross-reactivity for numerous other PCHs (Table 1).

The fiber optic immunosensor detected Aroclor 1242 with a sensitivity limit of 10 ng/mL (Figure 11). Recoveries of Aroclor 1242 from river and bay waters and from extracts of SRM soils were reasonably good. They ranged from 71 to 114% for the water samples and from 79 to 119% for soil extracts, with the exception of the two SRM lower concentrations (Tables 2 and 3). The displacement curves of both Aroclor 1242 and 1248 were shifted approximately 1 order of magnitude to the right (i.e. higher EC_{50} values) as compared to ELISA assays using the same anti-PCB Abs (Johnson and Van Emon, 1994). This is an interesting observation in light of two previous reports that two other fiber optic immunosensors detected parathion (Anis et al., 1991) and imazethapyr (Anis et al., 1993) with similar or lower detection limits than ELISA. Detection of PCBs may be improved by using a combination taper fiber which was described by Ligler and co-workers (Anderson et al., 1993). The taper fiber improves signal acquisition, enhances sensitivity, and improves reproducibility.

It has also been reported that polyclonal antiserum has been used to construct an ELISA suitable for field use. This field kit detected several Aroclors including Aroclor 1248 in soil samples at concentrations higher than 5 ppm (Mapes et al., 1993). The fluorescent immunosensor described in the present study detects TCPB and Aroclor 1248 at 10 ppb, which is 3 orders of magnitude higher sensitivity than that reported by Mapes et al. (1993) and is comparable to the detection limits of GC-based methods for measurement of PCBs as a class.

Evanescent fluorometry allows the observation of an immunochemical reaction in real time. Furthermore, the fact that initial rate fluorescence measurements parallel changes in the steady state fluorescence (Figures 4 and 5) suggests that one may use shorter assay times (e.g. 30 s) to measure Aroclors than were employed in the reported data. This strategy was applied successfully for detecting imazethapyr in soil extracts (Anis et al., 1993) and reflects a general advantage for biosensors.

At least two populations of immobilized Ab binding sites were observed to contribute to the biosensor-detected binding event. This may reflect the use of polyclonal Abs or arise from immobilization artifacts. This question may be resolved by production of a monoclonal Ab with optimal affinity for the analyte and its use to construct a specific immunosensor. The isolation of mRNA from the spleen of a mouse immunized with TCPB, followed by the production of reciprocal genes and cloning of Fab fragments into *Escherichia coli* (Ward, 1992), would provide an excellent means for selecting an Ab with optimal specificity for an analyte. This method would also guarantee a stable continuous supply of homogeneous Abs for the biosensor. The similarities in chemical structures of PCBs and the variety of their mixtures in the environment suggest the need for production of several monoclonal PCB Abs for the biosensor, each with high selectivity for a single PCB of interest. To minimize false positive results, the detection of each analyte may require the use of at least two Abs with different selectivity profiles.

LITERATURE CITED

Albro, P. W.; Luster, K. I.; Chae, K.; Chaudhary, S. K.; Clark, G.; Lawson, L. D.; Corbett, J. R.; McKinney, J. D. A radioimmunoassay for chlorinated dibenzo-p-dioxins. *Toxicol. Appl. Pharmacol.* **1979**, *50*, 137–146.

Anderson, G. P.; Golden, J. P.; Ligler. A fiber optic biosensor: combination tapered fibers designed for improved signal acquisition. *Biosens. Bioelectron.* **1993**, *8*, 249–256.

Anis, N. A.; Wright, J.; Rogers, K. R.; Thompson, R. G.; Valdes, J. J.; Eldefrawi, M. E. A fiber optic immunosensor for detecting parathion. *Anal. Lett.* **1992**, *25*, 627–635.

Anis, N. A.; Eldefrawi, M. E.; Wong, R. B. A reusable fiber optic immunosensor for rapid detection of imazethapyr herbicides *J. Agric. Food Chem.* **1993**, *41*, 843–848.

Baker, E. L.; Landrigan, P. J.; Glueck, C. J.; Zack, M. M.; Liddle, J. A.; Burse, V. W.; Houseworth, W. J.; Needham, L. L. Metabolic consequences of exposure to polycholinated biphenyls (PCBs) in sewage sludge. *Am. J. Epidemiol.* **1980**, *112*, 553–563.

Block, M. J.; Hirschfeld, T. B. Apparatus including optical fiber for fluorescence immunoassay. U.S. Pat. 4,582,809, 1986.

Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of an inhibitor that causes a 50% inhibition (I_{50}) of an enzyme reaction. *Biochem. Pharmacol. J.* **1973**, *22*, 3099–4106.

Devine, P. J.; Anis, N. A.; Wright, J.; Kim, S.; Eldefrawi, A. T.; Eldefrawi, M. E. A fiber-optic cocaine biosensor. *Anal. Biochem.* **1995**, *227*, 216–224.

Eldefrawi, M. E.; Eldefrawi, A. T.; Rogers, K. R.; Valdes, J. J. Pharmacological biosensors. In *Immunochemical Assays and Biosensor Technology for the 1990s*; Nakamura, R. M., Kasahara, Rechnitz, G. A., Eds.; American Society for Microbiology: Washington, DC 1992; pp 391–406.

Erickson, M. D. *Analytical Chemistry of PCBs;* Butterworth: Boston, 1986.

Fernando, J. C.; Rogers, K. M.; Anis, N. A.; Valdes, J. J.; Thompson, R. G.; Eldefrawi, A. T.; Eldefrawi, M. E. Rapid detection of anticholinesterase insecticides by a reusable light addressable potentiometric biosensor. *J. Agric. Food Chem.* **1993**, *41*, 511–516.

Goldberg, M. E.; Djavadi-Ohaniance, L. Methods for measurement of antibody/antigen affinity based on ELISA and RIA. *Curr. Opin. Immunol.* **1993**, *5*, 278–281.

Hatano, Y.; Hatano, A. Influence of polychlorinated biphenyls on the growth of chicken embryos. *J. Toxicol. Environ. Health* **1994**, *42*, 357–364.

James, R. C.; Busch, H.; Tamburro, C. H.; Roberts, S. M.; Schell, J. D. Harbison, R. D. Polychlorinated biphenyl exposure and human disease. *J. Occup. Med.* **1993**, *35*, 136–148.

Jensen, A. A. Background levels in humans. In *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins, and Related Products*, 2nd ed.; Kimbrough, R. D., Jensen, A. A., Eds.; Elsevier: Amsterdam, 1989; pp 245–380.

Johansen, H. R.; Becher, G.; Polder, A.; Skaare, J. V. Cogener-specific determination of polychlorinated biphenyls in human milk from Norwegian mothers living in Oslo. *J. Toxicol. Environ. Health* **1994**, *42*, 157–177.

Johnson, J. C.; Van Emon, J. M. Development and evaluation of a quantitative enzyme linked immunosorbent assay (ELISA) for polychlorinated biphenyls. U.S. EPA, ORD Report, EPA/600/R-94/112, June 1994.

Kreiss, K.; Zack, M.; Kimbrough, R. D.; Needham, L.; Smrek, A. L.; Jones, B. T. Association of blood pressure and polychlorinated biphenyls. *J. Am. Med. Assoc.* **1981**, *245*,-2505–2509.

Kutz, F. W.; Cook, B. T.; Carter-Pokras, O. D.; Brody, D.; Murphy R. S. Selected pesticide residues and metabolites in urine from a survey of the U.S. general population. *J. Toxicol. Environ. Health* **1992**, *37*, 277–292.

Lambert, G. H.; Hsu, C. C. Polyhalogenated biphenyl and the developing human. In *Similarities and Differences Between Children and Adults: Implication for Risk Assessment;* Guzelian, P. S., Henry, C. G., Olin, S. S., Eds.; ELSI Press: Washington, DC, 1992; pp 172–190.

Limbird, L. E. *Cell Surface Receptors: A Short Course on Theory and Methods*; Martinus Nijhoff: Boston, 1987.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent B. *J. Biol. Chem.* **1951**, *193*, 265–275.

Mapes, J. P.; McKenzie, K. D.; Stewart, T. N.; McClelland, L. E.; Studebaker, W. B.; Manning, W. B.; Friedman, S. B. An on-site immunoassay for determining PCB in soil. *Bull. Environ. Contam. Toxicol.* **1993**, *50*, 219–225.

McFarland, V. A.; Clarke, J. U. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. *Environ. Health Prespect.* **1989**, *81*, 225–239.

Mullin, M.; Sawka, G.; Safe, L.; McCrindle, S.; Safe, S. Synthesis of the octa-and nonachlorobiphenyl isomers and congeners and their quantitation in commercial polychlorinated biphenyls and identification in human breast milk. *J. Anal. Toxicol.* **1981**, *5*, 138–142.

Newsome, W. H.; Schields, J. B. Radioimmunoassay of PCBs in milk and blood *Int. J. Environ. Anal. Chem.* **1981**, *10*, 295–304.

Oroszlan, P.; Duveneck, G. L.; Ehrat, M.; Widmer, H. M. Fiber optic atrazine immunosensor. *Sens. Actuators* **1993**, *11*, 301–305.

Rogan, W. J.; Gladen, B. C. Neurotoxicology of PCBs and related compounds. *Neurotoxicology* **1992**, *13*, 27–36.

Rogers, K. R.; Valdes, J. J.; Eldefrawi, M. E. Acetylcholine receptor fiber optic evanescent fluorescent fluorosensor. *Anal. Biochem.* **1989**, *182*, 353–359.

Rogers, K. R.; Eldefrawi, M. E.; Menking, D. E.; Thompson, R. G.; Valdes, J. J. Pharmacological specificity of a nicotinic acetylcholine receptor optical sensor. *Biosens. Bioelectron.* **1991a**, *6*, 507–516.

Rogers, K. R.; Cao, C. J.; Valdes, J. J. Eldefrawi, A. T.; Eldefrawi, M. E. Acetylcholinesterase fiber optic biosensor for detection of anticholinesterases. *Fundam. Appl. Toxicol.* **1991b**, *16*, 810–820.

Rogers, K. R.; Fernando, J. C.; Thompson, R. G.; Valdes, J. J.; Eldefrawi, M. E. Detection of nicotinic receptor ligands with a light addressable potentiometric sensor. *Anal. Biochem.* **1992**, *202*, 111–116.

Safe, S.; Safe, L.; Mullin, M. Polychlorinated biphenyls: congener-specific analysis of a commercial mixture and human milk extract. *J. Agric. Food Chem.* **1985**, *33*, 24–29.

Schwalbe-Fehl, M. Immunoassays in environmental analytical chemistry. *Int. J. Environ. Chem.* **1986**, *26*, 295–304.

Sinks, T.; Steele, G.; Smith, A. B.; Watkins, K.; Shults, R. A. Mortality among workers exposed to polychlorinated biphenyls. *Am. J. Epidemiol.* **1992**, *136*, 389–398.

Ward, S. E. Antibody engineering: the use of *Escherichia coli* as an expression host. *FASEB J.* **1992**, *6*, 2422–2427.

Wong, R. B.; Anis, N.; Eldefrawi, M. E. Reusable fiber-optic-based immunosensor for rapid detection of imazethapyr herbicide. *Anal. Chim. Acta* **1993**, *320*, 141–147.

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