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A Monoclonal Immunoassay for the Coplanar Polychlorinated Biphenyls

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Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants with diverse toxic, teratogenic, reproductive, immunotoxic, and tumorigenic effects. Three of the least abundant of the 209 PCB isomers (congeners) are the most toxic and most difficult to quantify. These are 3,4,3′,4′-tetrachlorobiphenyl, 3,4,3′,4′,5′-pentachlorobiphenyl, and 3,4,5,3′,4′,5′-hexachlorobiphenyl (IUPAC No. 77, 126, and 169, respectively). An immunizing hapten was designed to retain the 3,4,3′,4′ chlorine-substitution pattern and coplanarity characteristic of these toxic congeners. The optimal competitors for immunoassay were weaker binding distinctive single-ring fragments of the PCBs. A monoclonal antibody designated S2B1 was derived and used in direct (antibody-capture) competitive enzyme immunoassays (EIAs). The EIAs are highly specific for non-ortho-substituted congeners and do not recognize the more prevalent but much less toxic noncoplanar PCB congeners or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, or dichlorobenzenes. Hapten and competitor design for this assay suggests a basis for development of sensitive EIAs for other classes of PCB congeners.

The polychlorinated biphenyls (PCBs) are among the most hazardous and ubiquitous man-made toxic compounds. They were in extremely wide use in numerous industrial applications from the 1930s until their toxicity, ability to bioaccumulate, and carcinogenic potential were recognized. Their manufacture was discontinued in the 1970s. PCBs are distributed so widely that they have been classified as global chemical pollutants. This group of compounds has from 1 to 10 chlorines on the biphenyl nucleus, with a total of 209 possible combinations (congeners). The chemical and physical properties of PCBs make analysis difficult. They are highly persistent, they adsorb to soils and colloidal materials, they leach very slowly, and they bioaccumulate up the food chain. Large amounts of these compounds remain in the environment, in use or in waste.

The toxicology and the carcinogenic, mutagenic, teratogenic, and immunotoxic properties of various congeners are detailed in an extensive literature. Recent volumes published by the U.N.—World Health Organization International Program on Chemical Safety are particularly comprehensive summaries of the current understanding of the distribution and toxicology of PCBs and polybrominated biphenyls (PBBs).[1,2] Some congeners and their metabolites have been implicated as estrogen mimics, with effects on postnatal development and reproductive ability.[3–5] The coplanar and noncoplanar PCBs differ in their toxicological properties. [The terminology in the literature is not consistent with the nuclear magnetic resonance (NMR) data. Coplanarity implies a dihedral angle of 0° between the phenyl rings. All PCBs tend to be noncoplanar to some extent in solution. The congeners referred to as "coplanar" in the literature are coplanar in crystals used for X-ray crystallography. These congeners have minimal, but nonzero dihedral angles in solution.] The three most toxic congeners, 3,4,3′,4′-tetrachlorobiphenyl (PCB 77), 3,4,3′,4′,5′-

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pentachlorobiphenyl (PCB 126), and 3,4,5,3′,4′,5′-hexachlorobiphenyl (PCB 169) are coplanar and structurally resemble dioxin.[6,7] These congeners are minor mole fractions of commercial PCB formulations.[1] Their primary mode of action is binding to the aryl hydrocarbon (Ah) receptor, which leads to induction of cytochrome P450-associated enzyme activities.[6,8,9] The much more abundant noncoplanar, ortho-chlorinated congeners have different mechanisms of toxicity that have not been well defined.

Analysis of PCBs is generally based on detecting the group of congeners that is most abundant in commercial formulations such as the Aroclors. There is increasing recognition that quantitation of the most toxic congeners is essential for evaluating the environmental impact of PCBs. However, gas chromatography of these congeners with electron capture detection (GC-ECD) or mass spectrometry (GC/MS) is particularly difficult because coeluting ortho-substituted congeners are typically present in much greater amounts.[7] Instrumental toxic congener analysis may cost as much as $1000 per sample. This greatly limits the scope of regulatory and research sampling.[10]

Congener-specific analysis has several advantages for regulatory as well as research applications. Commercial formulations each have relatively consistent mole fractions of certain congeners that provide a distinctive "signature". Draper[11] demonstrated that most of the Aroclor mixtures could be identified by capillary GC analysis of only 12 congeners. Nine congeners were classified as the most hazardous by McFarland and Clarke.[9] Thirty to fifty congeners are found in various tissue samples, but fewer predominate.[9,12] Canadian regulatory agencies use a reference mixture of 51 congeners for capillary GC analysis.[13] A series of indicator congeners for contamination of foods has been proposed.[14] The World Health Organization has emphasized the continuing need for long-term studies of the toxicity, epidemiology, and mechanisms of action of specific congeners and the value of identifying sensitive and specific biomarkers for some of the more subtle aspects of PCB toxicity.[15] Monoclonal antibodies and an immunoassay specific for the toxic congeners could be particularly valuable for this type of research. They could be used as independent screening methods or in conjunction with instrumental analysis.

The molecular heterogeneity, low aqueous solubility, lack of functional groups for derivatization, and other chemical properties of PCBs make design of immunoassays a daunting problem. All of the published PCB immunoassays we are aware of were designed to detect "Aroclor equivalents" or the most abundant noncoplanar PCB congeners.[16–21] To date we have not found any published reports of *monoclonal* antibodies for sensitive detection of PCB congeners or mixtures. The design and performance criteria for a toxic PCB congener immunoassay are exceptionally demanding. The assay must perform with sufficient sensitivity, accuracy, and precision despite the extremely low water solubility of the compounds. It must be specific for the coplanar congeners. There should be no significant cross-reaction with other halogenated biphenyls, dibenzofurans, dioxins, halowax (chlorinated naphthalene), or single-ring halogenated compounds, including chlorinated benzenes and phenols that may be present with PCBs in hazardous waste. Compounds such as DDT, DDE, and chlorophenoxy herbicides should not be recognized.

The immunizing hapten and competitor reagents for this project were synthesized expressly to derive a specific antibody and a sensitive assay for the most toxic PCB congeners. Our approach was based on the hypothesis that we could design an immunizing hapten to evoke high-affinity antibodies to the coplanar PCB structure, while molecules designed to mimic half of the PCB could serve as competitors of lower binding affinity in competition immunoassays. In the course of this work it became evident that polyclonal antisera would be unlikely to provide the specificity or sensitivity needed for single congener analysis and that monoclonal antibodies (MAbs) would be required.

This paper describes direct enzyme immunoassays (EIAs) that are highly selective for PCBs 77 and 126, utilizing one MAb developed with this strategy. An EIA using tubes coated with a capture antibody gives the most sensitive limit of detection. However, a format using streptavidin-coated microwells to capture biotinylated MAb is more reproducible for samples in more than 5% organic solvent. In 5% methanol, only PCBs 77 and 126 are recognized with a limit of detection at or below 1 ppb. Other congeners are less than 3% cross-reactive. In 10% DMSO, five ortho-substituted congeners are detected with I_{50} values less than 100 ppb, and five others including PCB 169 react with I_{50} values of 100–500 ppb. Mono-ortho-chlorination reduces or eliminates binding and di-ortho-chlorinated PCBs are not bound.

EXPERIMENTAL SECTION

Reagents and Materials. All reagents were purchased from Fisher Scientific, Aldrich Chemical Co., or Sigma Chemical Co. unless otherwise indicated. Only deionized, glass-distilled water was used, and Spectrograde methanol, DMSO, and 2-propanol were used as PCB solvents. PCB congeners >99% pure and all

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other reference standards were purchased from AccuStandard, Inc. (New Haven, CT). Reference solutions of 200 ppm were prepared in 2-propanol and stored at 4 °C in glass vials with Teflon-lined screw caps.

Thin-layer chromatography (TLC) was performed on Analtech 250-μm silica gel GF Uniplates. Flash column chromatography was done with hand-packed 40 μm silica gel columns. Gas chromatography (GC) was performed on a Hewlett-Packard 5890 system equipped with an FID detector and a 30 m × 0.32 mm i.d. Supelco SPB-5 (0.25-μm film of 5% diphenyl-, 94% dimethyl-, 1% vinylpolysiloxane) column. The following GC conditions were used: initial temperature 100 °C; temperature hold 2 min, then 15 °C/min to 275 °C. NMR spectra were recorded with Nicolet NT-300 or IBM 200 MHz instrument. Mass spectra were typically obtained with an AEI-ms 30 spectrometer.

Swiss Webster mice were purchased from Simonsen Laboratories (Gilroy, CA), and Biozzi and B10.Q mice were from stock bred in the U.C. Berkeley Hybridoma Facility mouse colony. Titermax adjuvant was purchased from Vaxcel, Inc. (Norcross, GA), and Ribi MPL+TDM Emulsion was from Ribi Immunochem Research, Inc. (Hamilton, MT). Cell culture medium was purchased from Grand Island Biological Co. (GIBCO-BRL, Grand Island, NY), antibiotics and other additives were from Sigma Chemical Co., and fetal bovine serum was from Intergen, Inc. (Kankakee, IL).

Indirect EIAs were performed in Immulon 2 microplates (Dynatech, Inc., Chantilly, VA). The Immunosystems division of Millipore Corp. (Scarborough, ME) provided tubes, 12-well microwell strips coated with donkey anti-mouse immunoglobulin, and a diluent for the PCB hapten–horseradish peroxidase (HRP) conjugates used in some direct EIAs. The peroxidase substrate was a stabilized single-component tetramethylbenzidine (TMB) formulation (Catalog No. 50-76-05, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Streptavidin-coated microwell strips were purchased from Labsystems Corp. (Needham Heights, MA). Bovine serum albumin (BSA) and alkaline phosphatase substrate tablets (*p*-nitrophenyl phosphate) for EIAs were purchased from Sigma Chemical Co. Alkaline phosphatase–antibody conjugates used for indirect EIAs were obtained from Boehringer-Mannheim Corp.

Safety Precautions. PCB reference standards were stored at 4 °C in glass vials with Teflon-lined screw caps. The vials were stored upright in a spill-proof steel box. All dilutions of PCBs were made in a chemical fume hood. PCBs were diluted into neat methanol in disposable glass tubes using a positive-displacement glass capillary pipettor with a Teflon plunger (Wheaton Corp., Millville, NJ). EIA steps involving solutions and rinses of microplates and tubes that contained PCBs were done in a stainless steel pan in a chemical fume hood lined with disposable paper. Solutions were aspirated into a glass waste container using a vacuum manifold (Nunc ImmunoWash 12). The vacuum line was protected with a glass trap and a Vacushield liquid-barrier filter. The analysts wore spill-resistant gowns and double nitrile gloves.

Chemical Syntheses. The immunizing hapten and competitor reagents used in this project are shown in Figure 1. The short names used in the text appear below the structures.

Hapten Synthesis. The immunizing hapten was synthesized by a Cadogan coupling of 3,4-dichloroaniline with 3-chloroanisole to form the three expected isomeric methoxytrichlorobiphenyls

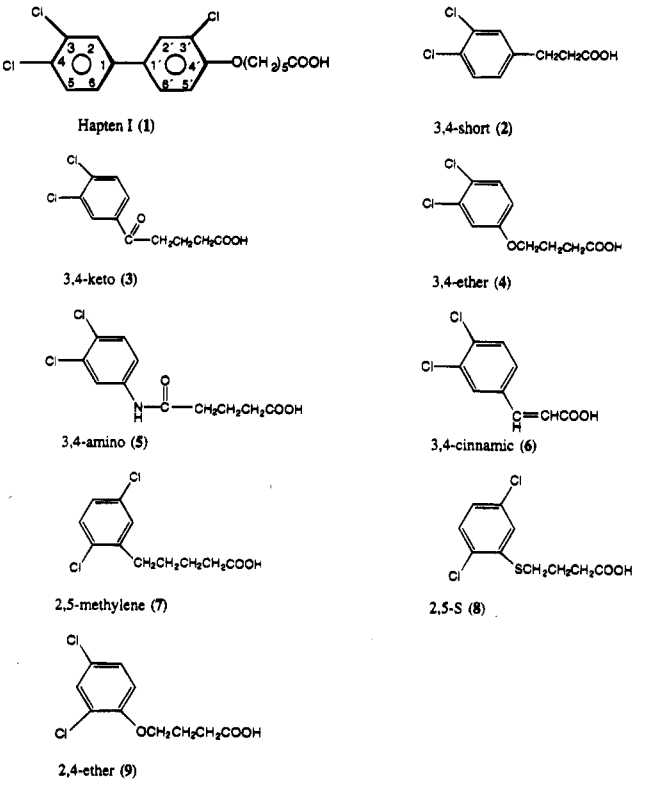

Figure 1. Immunizing hapten and competitors used in this study.

(**10**).[22] These were distinguished by NMR and resolved by GC-FID. Isomer **10c** was readily separated from the crude product by flash chromatography. Isomers **10a**, **10b**, and **10d**, obtained as a difficult to resolve mixture, were carried into BBr_3 demethylation. The desired biphenylol (**11**) was obtained by flash chromatography. **11** was alkylated with ethyl 6-bromohexanoate to yield the ethyl ester **12**. Upon isolation of the pure ester precursor, hapten I (**13**) was prepared by LiOH hydrolysis of **12** at room temperature (Figure 2). All intermediates and the hapten were characterized for purity by TLC and by gas chromatography with a flame ionization detector (GC-FID). Structures were verified by NMR and mass spectrometry (MS).

3,3′,4′-Trichloro-4-hydroxybiphenyl. Isoamyl nitrite (2.69 mL, 20 mmol) was added portionwise over the course of 10 min to a mixture of 2-chloroanisole (10.7 mL, 80 mmol) and 3,4-dichloroaniline (1.62 g, 10 mmol) at 120 °C under nitrogen, and the reaction was allowed to stir for 18 h. The excess anisole was distilled under vacuum to give a residue which was purified by flash chromatography (silica gel, 95/5 petroleum ether/methylene chloride eluant). The major fraction (TLC R_f = 0.60; minor fraction R_f = 0.76) was concentrated to a residue by removal of the solvent on a rotary evaporator. The minor fraction had a GC retention time of 14.5 min; the major fraction, a GC retention time of 15.4, 16.0, 16.2 min. The major fraction residue was dissolved in 10 mL of methylene chloride, and 4 mL of 1 M BBr_3 in methylene chloride was added. The reaction was stirred under nitrogen at room temperature for 24 h and worked up by the addition of approximately 10 mL of saturated potassium dihydrogen phosphate followed by removal of the aqueous layer and addition of anhydrous sodium sulfate to the methylene chloride fraction. Flash chromatography (silica gel, 95/5 petroleum ether/

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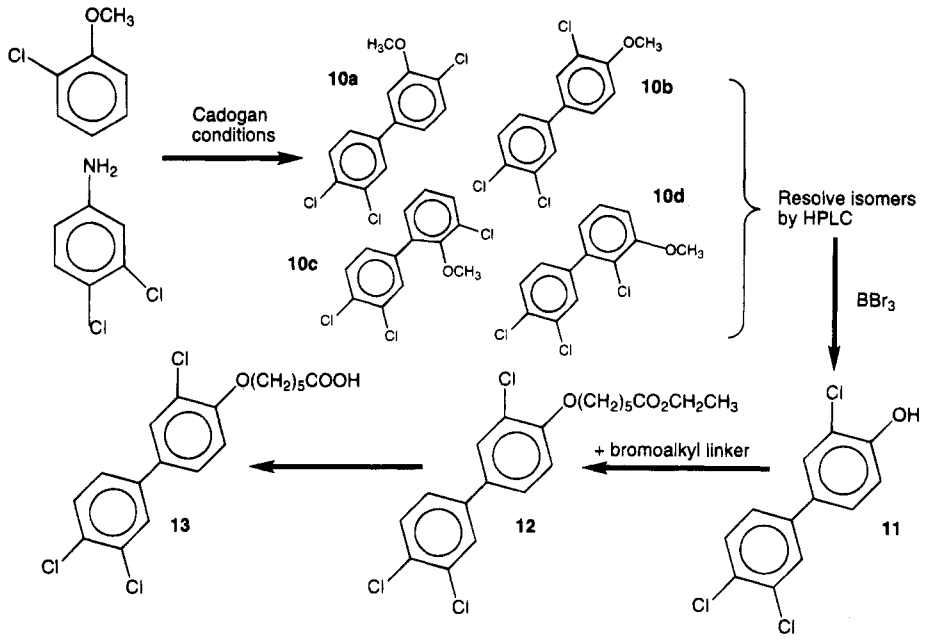

Figure 2. Synthesis scheme for the immunizing hapten.

ethyl acetate) of the residue obtained after removal of the solvent gave 91 mg (3.3% based on 3,4-dichloroaniline) of the desired compound **(11)** as a white solid: TLC (petroleum ether/ethyl acetate, 95/5) $R_f = 0.44$; ^{1}H NMR (200 MHz, $CDCl_3$) $\delta = 7.58$ (d, $J = 2.1$ Hz, H2′), 7.50 (d, $J = 2.3$ Hz, H2), 7.48 (d, $J = 8.5$ Hz, H5′), 7.35 (dd, $J = 8.5$, 2.2 Hz, H6′), 7.32 (dd, $J = 8.4$, 2.3 Hz, H6), 7.08 (d, $J = 8.4$ Hz, H5).

6-[(3,3′,4′-Trichlorobiphenyl-4-yl)oxy]hexanoic Acid (13, Hapten I). Ethyl 6-bromohexanoate (65 μL, 0.36 mmol) was added to a solution of the biphenylol (**11**; 91 mg, 0.33 mmol) in 15 mL of acetone. Anhydrous potassium carbonate (55 mg, 0.40 mmol) and potassium iodide (5 mg) were added, and the mixture was refluxed for 18 h. The reaction solution was filtered and evaporated to dryness to yield a crude residue. To this residue was added 6 mL of absolute ethanol and 1.25 mL of 1 N LiOH. The reaction was stirred at room temperature overnight. On addition of 1.0 mL of 1 N HCl, the product was obtained as a microcrystalline powder (88 mg, 69%): TLC (petroleum ether/ethyl acetate, 95/5 with 0.1% acetic acid) $R_f = 0.57$; ^{1}H NMR (300 MHz, CD_3OD) $\delta = 7.66$ (d, $J = 2.1$ Hz, H2′), 7.59 (d, $J = 2.3$ Hz, H2), 7.52 (d, $J = 8.3$ Hz, H5′), 7.46 (dd, $J = 8.3$, 2.1 Hz H6′), 7.43 (dd, $J = 2.3$, 8.6 Hz, H6), 7.07 (d, $J = 8.6$ Hz, H5), 4.09 (t, $J = 6.2$ Hz, phenyl-OCH_2), 2.35 (t, $J = 7.1$ Hz, CH_2COOH), 1.93–1.83 (m, phenyl-OCH$_2$CH_2), 1.78–1.68 (m, CH_2CH$_2$COOH), 1.64–1.53 (m, CH$_2$CH_2CH$_2$); MS (EI, *m/z* (relative intensity)) 388 (11), 386 (M^+, 12), 276 (29), 274 (94), 272 (100). High-resolution MS (EI, 70 eV), $C_{18}H_{17}Cl_3O_3$ requires 386.0231, found 386.0237.

Conjugation of Hapten I. Hapten I was conjugated to the carrier proteins keyhole limpet hemocyanin and bovine serum albumin by a standard activation of the hapten's carboxyl group with *N*-hydroxysuccinimide and carbodiimide in dimethylformamide.[23,24] The activated hapten was conjugated to the carrier protein in a borate buffer at pH 9.2. Control reactions, which contained hapten and protein without the activating agent, were used to evaluate the efficiency of the aqueous 2-propanol dialysis procedure for removal of noncovalent hapten from the conjugate solution (vide infra).

Hapten I, calculated to be a 200-fold molar excess over keyhole limpet hemocyanin (KLH) or a 100-fold molar excess over BSA, was dissolved in 400 μL of dimethylformamide (Aldrich, gold label) and activated to form the *N*-hydroxysuccinimide (NHS) ester. The activation was carried out using a 1.4-fold molar excess (calculated over the hapten) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) and a 2-fold molar excess (calculated over the hapten) of NHS added dry to the hapten solution. The carrier protein was dissolved in borate buffer (0.1 M, pH 9.4) to a final concentration of 10 mg/mL. The protein solution was allowed to stir overnight at 0–5 °C to ensure that all of the protein was dissolved. Dimethylformamide (Aldrich, gold label) was added to a concentration of 5% (v/v). The activated hapten solution was added to the protein solution, 10 μL at a time, every 30–60 min, using a 10-μL pipettor. The conjugation mixtures were allowed to stir overnight at room temperature after the hapten solution had been added. The reaction solutions were transferred to wetted cellulose dialysis tubing (MW cutoff 12 000–14 000) and dialyzed vs two changes of 0.5–1.0-L volumes of 10% (v/v) 2-propanol in phosphate-buffered saline (PBS, pH 7.4) over 2 days. Controls with EDC omitted showed that this method removed all nonspecifically bound hapten from the carrier protein. The reaction solutions were then dialyzed vs two changes of 0.5–1.0-L volumes of PBS over 2 days to remove any traces of 2-propanol. After dialysis, the conjugate solutions were collected from the dialysis tubing.

Load Determination. The moles of carrier protein were determined by the Lowry method with the use of an appropriate standard curve.[25] The moles of hapten were determined using UV/visible spectroscopy of the conjugate and the UV/visible spectrum of the hapten. The conjugate load was determined by

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dividing the moles of hapten present by the moles of carrier protein (KLH, 300 000 daltons; BSA, 67 000 daltons).

The KLH conjugate used for immunization had an average of 89 mol of hapten I/mol of KLH. No hapten association with KLH was detected in a control reaction that omitted activating agent. The BSA conjugate used for indirect EIAs had approximately 48 mol of hapten I/mol of protein, with 2.2 mol/mol of nonspecific association in the absence of activating agent. Hapten I–KLH and hapten I–BSA both tended to aggregate when stored at 4 °C for several weeks, possibly due to the high hapten load. Aliquots were clarified by centrifugation (12 000*g*, 5 min), and only soluble conjugate was used for immunizations and EIAs.

Synthesis of Competitors. The syntheses involved addition of a linker moiety by alkylation or acylation to an appropriate chlorophenyl synthon, similar to the conversion of **11** to **13** in Figure 2. Competitor **2** (3,4-short) was purchased from Trans-World Chemicals (Kensington, MD). Preparation of the 3,4-keto competitor **3** utilized the Friedel–Crafts acylation of *o*-dichlorobenzene with glutaric anhydride, essentially as described by Rosowsky.[26] The precursor compounds were all commercially available. Competitor **4** (3,4-ether) was prepared as described by Tandon et al.[27] Competitor **5** (3,4-amino) was prepared as described by Rashid.[28] Competitor **6** (3,4-cinnamic) was purchased from Aldrich Chemical Co. (St. Louis, MO). Synthesis of competitor **7** (2,5-methylene) was described elsewhere.[29] Competitor **8** (2,5-S) was prepared as reported by Kukalenko,[30] and competitor **9** (2,4-ether) was purchased from Aldrich Chemical Co. Competitor structure and purity were determined by TLC, GC-FID, UV/visible spectrophotometry, NMR, and/or MS.

Synthesis of Competitor–BSA and Peroxidase Conjugates. Competitors **2–9** were conjugated to BSA or amino-modified HRP using the carbodiimide-mediated carboxyl activation procedure described above to couple hapten I to BSA and KLH. HRP conjugates of hapten I were also prepared. The HRP (Catalog No. P-6782, Sigma Chemical Co.) was modified as described by Hsiao[31] to give 6–24 free amines. The conjugate stock solutions typically had a concentration of 5–10 mg/mL HRP, and they were stored at 4 °C.

Preparation of Biotinylated MAb. Immunoglobulin from MAb S2B1 (IgG) was purified to near-homogeneity by affinity chromatography of ascites fluid on protein A-Sepharose using a 1-mL HiTrap column (Pharmacia Biotech, Piscataway, NJ). The IgG was dialyzed overnight against three changes of 0.02 M KPO_4 (pH 7)/0.01 M EDTA/0.05 M NaCl. Protein concentration was determined by absorbance at 280 nm. A 1-mg sample of a biotin-spacer arm ester (NHS-XX-biotin; Calbiochem, La Jolla, CA) was dissolved in 0.5 mL of DMSO. Approximately 25 ng of ester was added in aliquots, with gentle shaking, to 1 mg of S2B1 IgG in 0.55 mL of 0.02 M Na_2CO_3/NaHCO3 (pH 9) in a 1 mL glass vial. After 2 h at room temperature the solution was dialyzed against three changes of the KPO_4/EDTA/NaCl buffer overnight. Aliquots were stored at −70 °C or as a 50% glycerol solution at −20 °C. The extent of biotinylation and ability to bind PCB–HRP competitor were determined by EIA.

Immunization and Monitoring of Mice. Four mice each of three strains (Swiss Webster, Biozzi, B10.Q) were immunized with the hapten I–KLH conjugate. The immunizing doses consisting of approximately 50 μg of conjugate (as carrier protein) in 0.08 mL of physiological saline, emulsified with one mouse dose of Titermax adjuvant (Vaxcel, Inc.), were delivered subcutaneously in three or four sites on the back of the mouse. Identical doses were given 7, 22, 106, and 133 days after the initial injection, except that Ribi adjuvant was used instead of Titermax on days 106 and 133. The adjuvant was changed because the Titermax adjuvant used in the first three injections had accumulated at the injection sites, and Ribi adjuvant is cleared from the sites. Serum samples were taken from the tail vein on days 29, 120, and 137. Anti-hapten titers were determined on day 29 serum by indirect EIA on wells coated with hapten I–BSA. All 12 mice developed a strong anti-hapten response (signal at serum dilutions >50 000). At this stage, four B10.Q and three S/W mice had developed a weak competitive binding response to PCB 77 as measured by indirect EIA with 2,5-S–BSA (competitor **8**). An indirect competition EIA with three of the sera taken on day 120 showed an improved competitive binding of 3,4,3′,4′-tetrachlorobiphenyl vs 2,5-S-BSA. However, the day 137 sera from only one mouse, a S/W, showed competitive binding responses specific for PCBs 77 and 126 in this assay. On day 154, 3 days prior to cell fusion, this mouse was given a subcutaneous boost with 100 μg of hapten I–KLH in Ribi adjuvant. To lessen the risk of anaphylactic or delayed-type hypersensitivity responses, this mouse received a subcutaneous injection of antihistamine and antivasospasm drugs 1 h before the boost.[32]

Hybridoma Production. All components of the cell culture media, electrical cell fusion procedures, and cryopreservation methods were as previously described.[33] Hybridoma colonies were screened by automated sampling between 12 and 18 days postfusion. Samples of 0.12 mL of supernate were transferred onto 96-well culture plates. Aliquots (0.05 mL) were transferred onto EIA plates coated with hapten I–BSA and plates coated with 2,5-S–BSA (competitor **8**) conjugate. A total of 628 colonies were screened in three groups. Of these, 161 reacted only with hapten I–BSA and 123 reacted with both hapten I–BSA and 2,5-S–BSA. None of the MAbs bound exclusively to 2,5-S–BSA. All 284 cultures were expanded to 24-well culture dishes. Two aliquots of each cell line were frozen and stored in liquid nitrogen. Culture supernates that bound hapten were tested for competitive binding of PCB congeners in direct EIAs as described in Results and Discussion. Only one MAb, designated S2B1, gave the desired results. This cell line was subcloned by limiting dilution, and 11 stable subclones were expanded and frozen. One subclone was expanded to produce culture medium and ascites. The ascites was prepared in irradiated Swiss Webster mice as described previously.[33] Immunoglobulin subclass was determined by EIA using a commercial kit (Southern Biotechnology Associates, Birmingham, AL).

Enzyme Immunoassay Methods. Indirect (immobilized competitor conjugate) and direct (immobilized antibody) EIAs

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used for evaluating responses of mice and for primary screening of MAbs utilized reagents and procedures of Voller *et al.*,[34] as modified in Karu et al.[33] Secondary screening and subsequent characterization of the MAbs was done by direct EIA using plastic 12 × 75 mm tubes or microwells coated with donkey anti-mouse IgG (DAM). Where indicated, the direct EIA was done with biotinylated MAb S2B1 captured on streptavidin coated microwells. Tubes and plates containing PCBs were handled as described above in Safety Precautions.

Indirect competition EIAs were performed by coating wells with amounts of hapten–BSA or competitor–BSA conjugate that were determined to be subsaturating by a checkerboard EIA. Wells were blocked with PBST–BSA.[33] Mixtures for competition (0.1 mL) containing a limiting dilution of antiserum or hybridoma culture fluid and PCB standards (0.01–5000 ppb, diluted in PBST–BSA) were incubated overnight at room temperature in sealed glass tubes. The competition mixtures were added to the blocked plates for 2 h at room temperature, the wells were washed, alkaline phosphatase conjugated goat anti-mouse IgG was added, and the remainder of the assay was performed as previously described.

Direct EIAs (soluble competitor–enzyme conjugate; immobilized antibody) were performed in 12 × 75 mm polystyrene tubes or microwell strips coated with donkey anti-mouse IgG. The same procedures and incubation intervals were used for the coated tube and microwell EIAs. The PBST used in these EIAs contained only 0.01% (w/v) Tween 20 because higher concentrations of Tween 20 reduced the sensitivity. The blocking reagent was 1% (w/v) BSA/0.05 g/mL sucrose in PBS. All dilutions of PCBs were first made into neat methanol, in glass tubes. Aliquots of these were taken into an "assay diluent" consisting of 0.005% Tween 20 in glass distilled water and methanol to give a final methanol concentration of 5%. These dilutions were made directly in the antibody-coated tubes or made in a glass tube and added to coated wells. Transfers were made using a positive-displacement glass capillary pipettor with a Teflon plunger. The diluent for PCB competitor–HRP conjugates was a proprietary solution obtained from the Immunosystems division of Millipore Corp. Stopping solutions for end-point assays were 1% HCl for tube EIAs and 2.5% HCl for microwell EIAs. The volumes used in each EIA are summarized below:

	volume added (mL)	
step	tube	microwell
coating with MAb	0.5	0.2
blocking	0.6	0.25
analyte (standard or sample) solution	0.51	0.2
competitor–HRP conjugate	0.2	0.2
HRP substrate (chromogen)	0.5	0.2
stop solution	0.5	0.05

Aliquots of sera or hybridoma culture supernates diluted in PBST (1:100 to 1:40000) were allowed to stand in the tubes or wells overnight at room temperature. The antibody solution was decanted and the tubes or wells were washed four times with distilled water. Nonspecific binding was prevented by addition of blocking buffer for 3–4 h at room temperature or overnight at 4 °C. The blocking buffer was decanted, and the tubes or wells could then be used immediately for EIA or they could be air-dried overnight at room temperature and stored for several weeks at 4 °C in Zip-lock bags.

The assay was performed by adding the PCB sample in assay diluent to the required number of antibody-coated tubes or wells and incubating 15 min at room temperature. The fluids were then aspirated into a waste reservoir, and the tubes/wells were rinsed four times with 0.5 mL of deionized water and shaken dry. Diluted PCB–HRP conjugate was then added for 5 min at room temperature. This solution was then aspirated and the tubes/wells were washed four times as before. Chromogen solution was then added to the tubes or wells. Color development was stopped by addition of HCl stopping solution. Aliquots of 0.1 mL were taken from tube EIAs into a microplate. Absorbance at 450 nm was recorded on a microplate reader. Nonspecific binding was determined using tubes or wells coated with nonimmune mouse serum or complete IMDM cell culture medium. Results were expressed as the ratio of B (A_{450} for the sample) to B_0 (that obtained with diluent containing no PCB). Competition EIA dose–response curves (B/B_0 vs log analyte concentration) were fitted using the four-parameter logistic model, and the data were analyzed as described previously.[35]

Direct EIAs were also performed in microwells purchased with covalently attached streptavidin (160 ng/well). Dilutions (7.5 ng/well) of biotinyl-S2B1 IgG in PBST were allowed to bind for 1 h at room temperature. The wells were washed, blocked for 30 min with PBST/0.01% gelatin, and the remainder of the assay was conducted as described above. Where indicated, the assay diluent was adjusted to methanol concentrations up to 25% (v/v) to take advantage of this format's solvent tolerance.

RESULTS AND DISCUSSION

Immunizing Hapten and Competitors. Design of the immunizing hapten to evoke toxic congener-specific antibodies was based on three considerations. First, we retained the 3,3′,4,4′ chlorine-substitution pattern and coplanarity that are characteristic of the most toxic congeners. Second, an ether was used as a chlorine mimic for attachment of the spacer arm to the biphenyl. Third, the ether moiety spacer was attached to the biphenyl at the para position. Use of the ether linkage and para substitution was previously successful in deriving antibodies specific for the noncoplanar PCBs.[29] Most previously published PCB haptens used an amide spacer which was attached at the ortho position. The cross-reaction of antibodies raised by those haptens suggested that steric differences and noncoplanarity result from ortho placement of the amide spacer. Mattingly[36] used several para-substituted linkers, but none with the 3,3′,4,4′-substitution pattern. Molecular models clearly showed that the para-substituted hapten used for this project (Figure 1) presents the coplanar biphenyl moiety extended distally from the linker. By contrast, ortho attachment places the linker moiety in a central position on the hapten.[37] Accordingly, we hypothesized that para orientation of the linker would lead to a combining site that would be more specific for the coplanar PCBs.

The competitors for EIA development were designed from different criteria. Our previous success developing an EIA for

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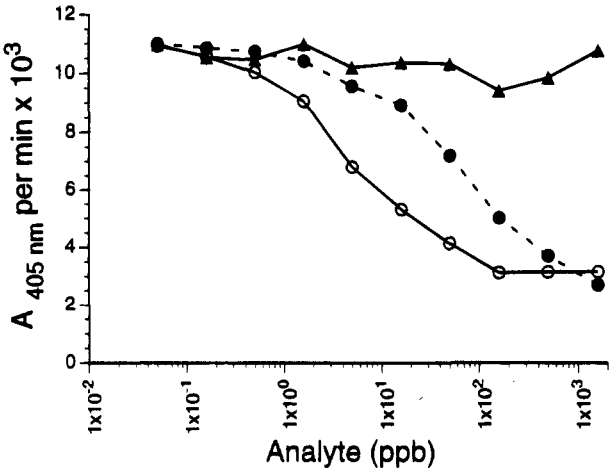

Figure 3. Indirect competition EIAs with mouse 2007-1 serum. Microwells were coated with 400 ng/well of the 2,5-S–BSA conjugate, and dilutions of the indicated PCBs were incubated with a 1/1667 dilution of 2007-1 serum from the third trial bleeding: (▲) 2,4,5,2′,4′,5′-hexachlorobiphenyl (PCB 153); (●) 3,4,3′,4′,5′-pentachlorobiphenyl (PCB 126); (○), 3,4,3′,4′-tetrachlorobiphenyl (PCB 77).

Table 1. Sensitivity of the Direct EIA for 3,3′,4,4′-Tetrachlorobiphenyl Using Mouse 2007-1 Serum and Various HRP Conjugates[a]

competitor (HRP conjugate)	$I_{50\ (ppb)}$ for PCB 77
2,5-S (**8**)	1.5
3,4-keto (**3**)	3
3,4-ether (**4**)	3.5
2,5-methylene (**7**)	6–8
hapten I (**1**)	30–40

[a] Numbers in parentheses refer to structures in Figure 1.

Table 2. Cross-Reactivity of PCB Congeners in the Direct EIA with Mouse 2007-1 Serum and the 3,4-Keto–HRP Competitor

IUPAC no.	congener	$I_{50\ (ppb)}$
77	3,4,3′,4′-tetrachlorobiphenyl	3
70	2,5,3′,4′-tetrachlorobiphenyl	50
118	2,4,5,3′,4′-pentachlorobiphenyl	290
15	4,4′-dichlorobiphenyl	4300
52	2,5,2′,5′-tetrachlorobiphenyl	>20000
153	2,4,5,2′,4′,5′-hexachlorobiphenyl	>20000

noncoplanar PCBs using PCB fragment-derived competitors suggested a similar approach for this assay.[37] However, we could not predict whether the most sensitive, congener-specific assay would result from antibody recognition of the most similar competitor or from less specific, lower affinity recognition of a dissimilar competitor. Differences in the pattern of competitor chlorination, linker placement relative to the chlorination pattern, and linker-to-aryl functional group produced different congener specificities in the EIA for noncoplanar PCBs (R. E. Carlson, unpublished). Consequently, in the present study we tested competitors with several different chlorination patterns, linker functional groups, and spacer lengths (Figure 1).

Responses of the Mice. By 29 days after the initial injection, all 12 mice produced sera with anti-hapten titers of >50 000. However, little or no competitive binding of PCB 77 was observed with the antisera at this stage. Sera taken from three of the mice 120 days after the initial dose showed competitive binding of PCBs 77 and 126 in EIAs using the 2,5-S competitor **8**. Serum from the best-responding mouse (Swiss Webster No. 2007-1) gave competitive binding of PCB 77 with an I_{50} less than 10 ppb, approximately 10-fold more sensitive than sera from the other two mice. The day 120 serum from mouse 2007-1 had less than 1% cross-reaction with 2,4,5,2′,4′,5′-hexachlorobiphenyl (PCB 153), which is a prevalent but relatively nontoxic Aroclor constituent, in indirect and direct EIAs with the 2,5-S competitor (Figure 3). The shallow inhibition curves suggested a large ensemble of antibodies had developed with widely differing affinities and specificities for the different analytes and competitors. Sera from the three mice that gave the best competitive binding with the 3,4-short competitor **2** also bound competitors **3**, **4**, **7**, and **8**. However, only mouse 2007-1 serum competitively bound the toxic congeners below 10 ppb, with no competitive binding of PCB 153.

The direct EIA with mouse 2007-1 day 120 serum was used to compare the HRP conjugates of the immunizing hapten and four competitors with PCB 77 as the analyte. The fragment-based competitors improved the sensitivity by 5-fold to 27-fold over that obtained with hapten I as the competitor (Table 1). The 3,4-keto competitor **3** gave a more sensitive and reproducible competition with PCBs 77 and 126 than the 3,4-short competitor **2** that was used in the earlier serum tests (data not shown). The results indicate that all of the fragment-based competitors improved the assay sensitivity. However, there was no clear relationship between the sensitivity and the competitor's chlorination pattern or linker moiety. The data in Table 2 demonstrate that direct EIA using the 3,4-keto competitor and 2007-1 antiserum showed preference for PCB 77 compared with congeners that are significantly more abundant components of the Aroclor mixtures. In a similar direct EIA with the mouse serum in antibody-coated tubes, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was not recognized in amounts up to 1 ppm.

Selection of Hybridomas. The fusion produced 628 hybridoma colonies from 3840 wells. On the basis of the results of the mouse serum testing, the culture supernatants were screened by indirect EIA for binding to hapten I–BSA or 2,5-S–BSA. A total of 284 cultures that produced hapten-binding antibodies were expanded to 24-well dishes. The secondary screening by direct EIA selected 69 MAbs able to bind hapten I–HRP, 2,5-S–HRP, or 3,4-keto–HRP. Only five of these competitively bound PCB 77 or showed relatively specific noncompetitive binding of the competitors. Of these, only one MAb, designated S2B1, proved to be specific for competitive binding of the toxic congeners PCB 77 and PCB 126. MAb S2B1 was an $IgG_{2b}\kappa$ immunoglobulin, which allowed us to affinity purify it on protein A-Sepharose.

Optimization of the Direct EIA. The direct EIA was optimized with respect to dilutions of MAb S2B1 culture fluid using competitors **2**–**9** (checkerboard titrations). Nonspecific binding of the competitor–HRP conjugate preparations was measured with tubes or wells coated with dilutions of complete cell culture medium (Iscove's Modified Dulbecco's medium, IMDM) instead of MAb S2B1. Each conjugate had different amounts of nonspecific binding. Adventitious binding of the 3,4-keto–HRP and 3,4-amino-HRP competitors was negligible at dilutions of IMDM greater than 1/500. The 2,5-S–HRP and the 2,4-ether–HRP conjugates bound so weakly that B_0 values were low, and nonspecific binding was higher than that of 3,4-keto–HRP. The 3,4-cinnamic acid–HRP conjugate had significant nonspecific binding

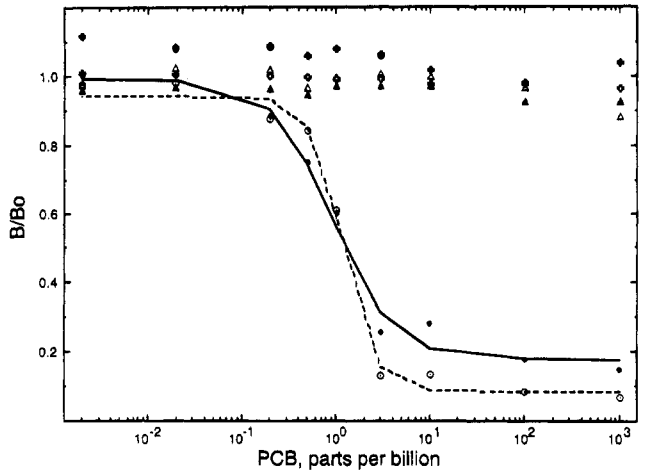

Figure 4. Direct competition EIA of six PCB congeners with MAb S2B1. Microwells coated with donkey anti-mouse IgG were allowed to bind S2B1 culture supernate (1/100 in PBST). Dilutions of the PCB congeners in 0.01 mL were added to 0.2 mL of a 1/15000 dilution of the 3,4-keto–HRP conjugate as described in the Experimental Section. Color development was stopped after approximately 2 min by addition of 0.05 mL of 2.5% HCl to the 0.2 mL of substrate solution, and absorbance was read at 450 nm. B/B_0 is the ratio of the absorbance at the indicated concentration of analyte to the absorbance obtained with no added analyte: (solid cross) 2,4′-dichlorobiphenyl (PCB 8); (open cross) 2,5,2′,5′-tetrachlorobiphenyl (PCB 52); (▲) 2,4,5,2′,4′,5′-hexachlorobiphenyl (PCB 153); (△) 2,4,4′-trichlorobiphenyl (PCB 28); (●) 3,4,3′,4′-tetrachlorobiphenyl (PCB 77); (○) 3,4,3′,4′,5′-pentachlorobiphenyl (PCB 126). The lines are four-parameter logistic fits of the data. The estimated I_{50} values were 0.9 and 1.2 ppb for PCBs 77 and 126, respectively.

at dilutions up to 1/10000, but it gave a similar I_{50} to the 3,4-keto–HRP conjugate. Direct competition EIAs using competitors **3**–**7** gave comparable I_{50} values for PCBs 77 and 126. These results suggest that the optimum competitors for MAb S2B1 have the same 3,4 chlorination pattern as the hapten, but a different linker moiety. The optimized direct EIA for competitive binding of the toxic congeners used microwells coated with a 1/300 dilution of S2B1 culture supernate and a 1/15000 dilution of 3,4-keto–HRP.

The specificity of the direct EIA in tubes and microwells was first tested with MAb S2B1 and the 3,4-keto–HRP conjugate using a sample diluent containing 5% methanol. Results for the microwell assay are illustrated in Figure 4. In two such experiments, the fitted I_{50} values were 0.9–2.7 ppb for PCB 77 and 1.2–3.7 ppb for PCB 126. None of the four noncoplanar, less toxic PCB congeners we tested bound competitively in amounts less than 2 ppm. The optimized coated tube EIA gave a similar result, with a minimum detection limit (I_{10}) of 0.2 ppb, an I_{50} of 1 ppb for PCB 77, and very similar values for PCB 126. None of the noncoplanar congeners that we tested were bound in amounts up to 1 ppm. Solvent tolerance experiments indicated that methanol concentrations less than 5% in the assay diluent may have been insufficient to keep more than 100 ppb of some congeners soluble. However, methanol concentrations of 7.5% or more increased outliers and error among replicates in the direct EIA using tubes and wells coated with donkey anti-mouse IgG.

Streptavidin–Biotin EIA. An alternate direct EIA format proved to be much more stable to organic solvents. Affinity-purified S2B1 IgG was labeled with biotin on a 14-carbon spacer arm. Optimal biotinylation was achieved with 25 ng of NHS-XX-biotin/mg of pure IgG. The optimum assay was obtained using wells with 160 ng of covalently attached streptavidin to bind 7.5 ng of biotin-S2B1/well, and 3,4-keto–HRP (diluted 1/100000) as competitor. The I_{50} values for PCBs 77 and 126 in this EIA were higher than those obtained in the direct EIA using capture antibody (7–30 ppb instead of 1 ppb), but they remained the same in 5, 10, and 25% methanol. The standard error of replicates was much lower in the biotin–streptavidin assay. This assay could also be performed using 10% DMSO rather than methanol in the diluent. This proved to be important for assays of dioxin, dibenzofuran, and other compounds that are poorly soluble in methanol. The specificity of MAb S2B1 for various congeners and other compounds and all experiments with Aroclors were done using the biotin–streptavidin EIA.

Table 3. Cross Section of MAb S2B1 with Various PCBs[a]

IUPAC no.	CAS no.	common name	I_{50}[b] (ppb) 5% methanol	I_{50}[b] (ppb) 10% DMSO
77	32598-13-3	3,4,3′,4′-tetrachloro	7–30	10–90
126	57465-28-8	3,4,5,3′,4′-pentachloro	10–20	50
169	32774-16-6	3,4,5,3′,4,′5′-hexachloro	nc[c]	370
12	2974-92-7	3,4-dichloro	≥2000	235
35	37680-69-6	3,4,3′-trichloro	310	20
37	38444-90-5	3,4,4′-trichloro	$I_{60} \approx 2000$[d]	135
70	32598-11-1	2,5,3′,4′-tetrachloro	>2000	600
78	70362-49-1	3,4,3′,5-tetrachloro	520	40
79	41464-48-6	3,4,3′,5′-tetrachloro	1500	65
81	70362-50-4	3,4,4′,5-tetrachloro	$I_{40} \approx 1000$[d]	~320
118	31508-00-6	2,4,5,3′,4′-pentachloro	1500	~5000

[a] The biotin–streptavidin EIA was performed with the 3,4-keto–HRP competitor as described in the Experimental Section. [b] Half-maximal inhibition determined by four-parameter logistic fit, unless noted otherwise. Columns show data obtained in diluent containing the indicated solvent. [c] nc, no competition in amounts up to 5 ppm. [d] Percent inhibition observed at the highest concentration tested. A four-parameter fit could not be computed where a high-dose asymptote was not observed.

Assay Specificity. The cross-reactivity of several congeners was strongly influenced by the amount and type of organic solvent in the assay diluent. Measurements of the three most toxic coplanar congeners (PCBs 77, 126, and 169) and the noncoplanar 2,4,5,2′,4′,5′-hexachlorobiphenyl (PCB 153) were compared in diluent containing 5, 10, or 20% methanol, DMSO, and acetonitrile. The responses of PCBs 77 and 126 were very similar, although the I_{50} values tended to increase (the assay became less sensitive) with increasing organic solvent. PCB 169 did not react in amounts up to 1 ppm in 5% methanol or 5% DMSO, and shallow binding curves were obtained in 10 and 20% methanol. In 10 and 20% DMSO, PCB 169 reacted with detection limits (I_{10}) around 100 and 10 ppb, respectively. Detection of PCB 169 in acetonitrile was similar to that in methanol. Based on these results, cross-reaction of several other congeners and analogs was tested in the biotin–streptavidin assay using PBST-10% DMSO as the diluent.

Table 3 compares the half-maximal or limiting inhibition by all of the congeners that showed appreciable binding in the EIA. Reactivity for most congeners was increased; i.e., the assay became more sensitive in diluent containing 10% DMSO. The assay using 10% DMSO was about equally sensitive for PCBs 77 and 126, but it also detected several other coplanar congeners with 3,4,3′ and 3,4,4′ chlorination. Two mono-ortho-substituted congeners (PCBs 70 and 118) bound weakly. All of the congeners in Table 3 were soluble in 5% methanol to at least 5 ppm. It appeared that the differences in detection were due to effects of

Table 4. Compounds That Are Weakly Reactive or Do Not Cross-React in the EIA[a]

IUPAC no.	CAS no.	common name	I_{50}[b] (ppb) 5% methanol	I_{50}[b] (ppb) 10% DMSO
		PCBs		
2	2051-61-8	3-chlorobiphenylol	nc[c]	nt[d]
8	34883-43-7	2,4′-dichloro	nc	nt
13	2974-90-5	3,4′-dichloro	nc	nt
14	34883-41-5	3,5-dichloro	nc	nt
15	2050-68-2	4,4′dichloro	nc	>3000
28	7012-37-5	2,4,4′-trichloro	nc	nt
52	35693-99-3	2,5,2′,5′-tetrachloro	nc	nt
56	41464-43-1	2,3,3′,4′-tetrachloro	nc	$I_{70} \approx 5000$[e]
76	70362-48-0	2′,3,4,5-tetrachloro	nc	$I_{40} \approx 3000$
80	33284-52-5	3,3′,5,5′-tetrachloro	nc	$I_{60} \approx 3000$
101	37680-73-2	2,4,5,2′,5′-pentachloro	nc	nt
105	32598-14-4	2,3,3′,4,4′-pentachloro	nc	$I_{35} \approx 5000$
110	38380-03-9	2,3,6,3′,4′-pentachloro	nc	nc
127	39635-33-1	3,3′,4,5,5′-pentachloro	nc	415
153	35065-27-1	2,4,5,2′,4′,5′-hexachloro	nc	nc
156	38380-08-4	2,3,3′,4,4′,5-hexachloro	nc	$I_{60} \approx 5000$
		PBBs		
	77102-82-0	3,4,3′,4′-tetrabromobiphenyl	$I_{40} \approx 1000$	~300
	77607-09-1	3,4,5,3′,4′,5′-hexabromo	nc	nc
	59080-40-9	2,4,5,2′,4′,5′-hexabromo	nc	nc
		Dibenzofurans and Dioxins		
	51207-31-9	2,3,7,8-tetrachlorodibenzofuran	*f*	$I_{15} \approx 5000$
	1746-01-6	2,3,7,8-tetrachlorodibenzo-*p*-dioxin	*f*	$I_{15} \approx 5000$
		PCB Metabolites		
		3,4′,5-trichloro-4-biphenylol	nc	nt
		3,3′,5,5′-tetrachloro-4,4′-biphenyldiol	nc	nt
		3,4,3′,4′-tetrachlorodiphenyl ether	*f*	$I_{15} \approx 1000$
		Other Compounds		
	95-50-1	1,2-dichlorobenzene	nc	nt
	541-73-1	1,3-dichlorobenzene	nc	nt
	106-46-7	1,4-dichlorobenzene	nc	nt
	120-82-1	1,2,4-trichlorobenzene	nc	nt
	95-76-1	3,4-dichloroaniline	nc	nt
	50-29-3	4,4′-DDT	nc	nt
	72-54-8	4,4′-DDD	nc	nt

[a] The biotin–streptavidin EIA was performed with the 3,4-keto–HRP competitor as described in the Experimental Section. [b] Half-maximal inhibition determined by four-parameter logistic fit, unless noted otherwise. Columns show data obtained in diluent containing the indicated solvent. [c] nc, no competition in amounts up to 5 ppm. [d] nt, not tested. [e] Percent inhibition observed at the highest concentration tested. A four-parameter fit could not be computed where a high-dose asymptote was not observed. [f] These compounds are not sufficiently soluble in PBST/5% methanol to conduct the assay.

DMSO on binding of the compounds by MAb S2B1 rather than to limited solubility of the congeners in methanol.

Table 4 lists compounds that were not reactive or reacted too weakly for practical detection. 3,4,3′,4′-Tetrabromobiphenyl, the brominated analog of PCB 77, bound weakly, suggesting that bromine atoms with their larger radii are not accommodated well in the combining site. The most toxic dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) and 2,3,7,8-tetrachlorodibenzofuran were not recognized in amounts up to 1 ppm; 5 ppm caused 15% inhibition at most. These compounds are approximate isostereomers of the PCBs.[6,38,39] The coplanar biphenylols and 3,3′,4,4′-tetrachlorodiphenyl ether are mammalian and microbial PCB metabolites with higher acute toxicity than the parent PCBs.[40,41] They also did not react in the EIA. None of several single-ring chlorinated compounds that may occur as PCB degradation products or are likely to be found with PCBs in hazardous waste were bound.

Similar selectivity but reduced sensitivity (higher I_{50} value) for PCBs 77 and 126 was observed in EIAs using hapten I–HRP and competitor–HRP conjugates **4**–**6** and **8** shown in Figure 2. This indicated that the specificity for the toxic coplanar congeners is an intrinsic property of MAb S2B1, due primarily to the immunizing hapten and not the competitors. These results also support the notion that the hapten defines specificity while the competitor determines the sensitivity of the assay.[37]

Detection of Aroclors. The Aroclors and related industrial PCB formulations vary greatly in their congener composition, including their content of the toxic coplanar tetra-, penta-, and hexachlorobiphenyls.[1] Several of the major Aroclors contained measurable amounts of congeners that are recognized in the assay. Direct EIAs were done with biotinyl-S2B1 in streptavidin-coated wells, to take advantage of the greater solvent tolerance. Aroclor stocks in neat methanol were diluted into PBST/25% methanol to give responses on the measurable range of a PCB 77 standard curve, based on published estimates of the mole

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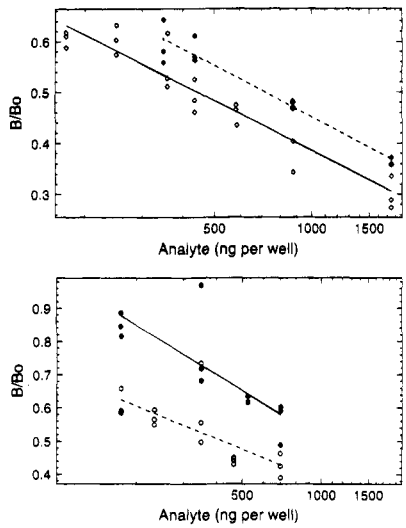

Figure 5. Measurement of immunoreactive material in Aroclors. The indicated amounts of Aroclor were added to the streptavidin–biotin EIA in diluent containing 25% methanol. Reference standards of PCB 77 had I_{50} values of 12–20 ppb, corresponding to 1.2–2 ng/well: (top) Aroclor 1248 (◆) and Aroclor 1016 (◇); (bottom) Aroclor 1242 (●) and Aroclor 1254 (○). The lines are logarithmic fits as described in the text.

percent of PCBs 77, 126, and 35 (Tables 1 and 2 in ref 1). Measurable responses were obtained from 0.06 to 1.75 μg of Aroclors 1016, 1242, 1248, and 1254 (Figure 5). Aroclor 1260 gave a very weak response ($B/B_0 = 0.7–0.9$ at 0.5–1.75 μg/well) with large variation between replicates. Three industrial polychlorinated terphenyls (Aroclors 5442, 5460, and 5060) gave no responses up to 2 μg/mL, nor did the polybrominated biphenyl formulation Firemaster BP-6.

The B/B_0 responses of Aroclors 1016, 1242, 1248, and 1254 and the pure congeners were fitted to the model $y = m \ln(x) + b$[42] using an iterative nonlinear fitting routine (Passage II). The responses were roughly parallel to each other over this range of Aroclor dilutions, but they were not parallel to the working ranges of curves for PCBs 77 and 126. These results probably reflect differences in the amounts of congeners that cross-react differently in the EIA and/or different nonspecific interfering substances in the Aroclors. Thus, while MAb S2B1 can detect the toxic congeners in Aroclors, it would not be accurate to estimate amounts of individual congeners by interpolation from a single-congener standard curve.

CONCLUSIONS

In summary, we have developed a MAb-based immunoassay that is selective for the most toxic PCB congeners. The selectivity is due primarily to the immunizing hapten with its coplanar structure and ether-linked, para-substituted spacer arm, while the sensitivity results from use of the fragment-based competitor–HRP conjugates. Previous efforts by others were designed to evoke antibodies that would recognize many of the more abundant PCB congeners. Thus, nearly all of the previously reported haptens were ortho-substituted and used an amino or azo linkage to mimic a chlorine atom.[16–18] Recently, PCB haptens with a glutaramyl-β-alanyl spacer arm were used to make an immunizing antigen that evoked polyclonal antibodies to PCB 153.[19,20] None of these assays were selective for the toxic coplanar congeners.

Most efforts at systematic hapten design have been based on the notion that both the immunizing hapten and the competitor should resemble the analyte as closely as possible.[43–45] However, competitive-binding immunoassays are generally most sensitive when the antibody has a lower affinity for the competitor than it does for the target analyte.[37,46] Accordingly, we designed our competitors to mimic half of the PCB molecule. The behavior of the mouse antisera as exemplified by Figure 3 indicated that our combination of immunizing and competing conjugates produced a congener-specific response. However, the results also suggested that the assay's performance might be limited by less selective or lower affinity antibodies in the repertoire. During hybridoma selection we also found that many antibodies in the repertoire bound to the immunizing hapten but did not competitively bind free PCB. Detection of individual PCB congeners appears to be an application for which MAbs are superior to whole antisera.

The limits of detection of PCBs 77 and 126 in our direct EIAs were 0.2–1.0 ppb, depending on the format. No immunoassay in the published literature reported a detection limit for these PCBs. The detection limit for these congeners in high-resolution capillary GC/MS is on the order of 10 ppb. Samples for CG/MS are generally concentrated 10 000-fold to give a detection limit around 1 (for extracts of fat) or 10–100 ppt (for extracts of sediments) in the original sample (Jianwen She and Kim Hooper, California Dept. of Health Services, personal communication). With similarly concentrated extracts, the EIA should thus be at least as sensitive as the instrumental method. Sample preparation for GC/MS also requires steps to eliminate noncoplanar PCBs that would coelute with the coplanar PCBs. These steps should not be necessary for the EIA.

Immunoanalysis could provide a more definitive and cost-effective way to identify and quantify the toxic congeners as an alternative to instrumental methods or in conjunction with them. The EIA is simple, fast, and amenable to automated sample processing. The negligible cross-reaction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran makes it possible to independently measure the coplanar PCB congeners in the presence of dioxins and dibenzofurans. This would not be possible using, for example, an assay based on the aryl hydrocarbon receptor. Although the EIA responds to a subset of congeners in the Aroclors, additional experiments will be needed to develop a reliable quantitative correlation between the EIA response and the toxic congener content of Aroclor-contaminated samples. Congener-specific immunoassay is also potentially applicable to environmental toxicology and molecular epidemiology studies. MAb S2B1 may prove to be a useful antagonist or receptor mimic in studies of PCB binding by proteins such as the aryl hydrocarbon receptor. In addition, immunoaffinity methods with MAb S2B1 may be suitable for specifically recover-

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ing residues of the toxic congeners from complex field samples. Our laboratories are presently exploring these and other applications.

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