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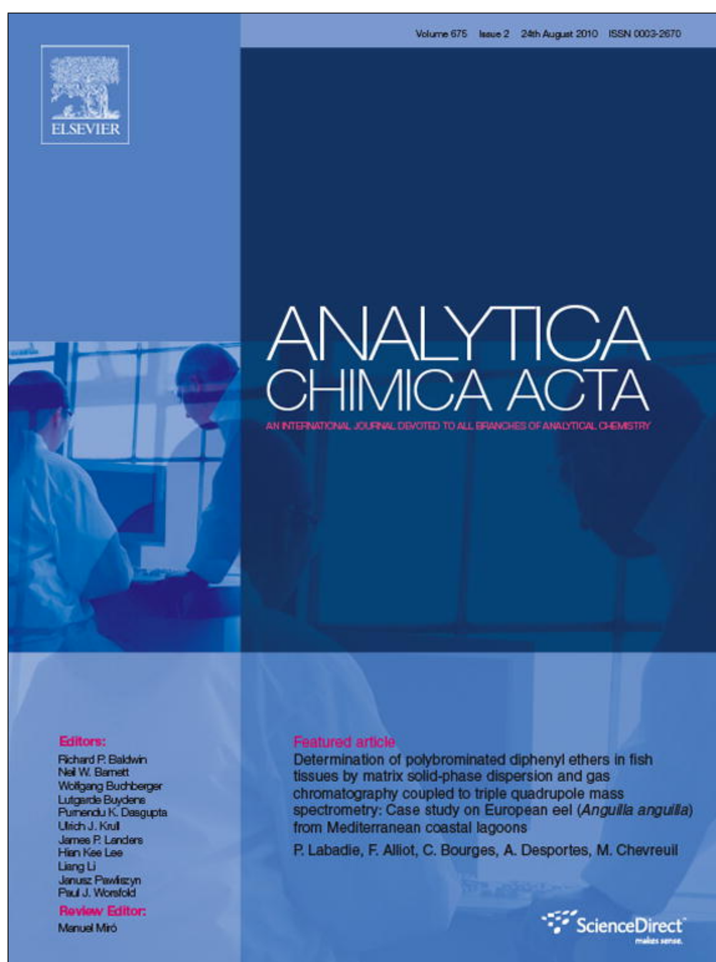

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Development of an immunoassay and a sol–gel-based immunoaffinity cleanup method for coplanar polychlorinated biphenyls from soil and sediment samples

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ABSTRACT

Two polychlorinated biphenyls (PCB) enzyme linked immunosorbent assays (ELISAs) were developed using goat PCB purified immunoglobulin (IgG) antibodies (Abs). The IgGs exhibited the highest affinity toward PCB-77 (24 ng mL^{-1}) with sensitivities in the range of 6–11 ng mL^{-1}. The Abs cross-reacted with PCB-126 and the heptachlorodibenzofuran 1,2,3,4,6,7,8-HpCDF but not with PCB-169, PCB-118, Aroclor 1232, 1248, 1260 or the hexachlorodibenzofuran 2,3,4,6,7,8-HxCDF. The IgGs were also used to develop a sol–gel-based immunoaffinity purification (IAP) method for cleanup of PCB-126. Recovery efficiencies depended on the sol–gel formats; a 1:12 format resulted in the highest binding capacity. Net binding capacity ranged from 112 to 257 ng, and 90% of the analyte could be eluted with only 2 mL of ethanol. The method was also very efficient in purifying PCB-126 from spiked soil and sediment samples from contaminated sites; and eliminating matrix interferences to a degree that enabled analysis of the purified samples by ELISA. The approaches developed in the course of the study form a basis for the development of additional IAP methods for other PCBs, and their implementation in high-throughput screening programs for PCB in food, soil, and other environmental and biological samples.

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1. Introduction

Polychlorinated biphenyls (PCBs) comprise 209 different compounds that share a common structure but vary in the number and position of attached chlorine atoms. PCBs are commonly used in capacitors and other electrical equipment because of their stability, insulating properties, and non-inflammability. Aroclors were manufactured as specific mixtures of PCB congeners. The manufacture of PCBs was banned in the U.S. in 1977 because of persistent accumulation in the environment, and harmful ecological and human effects. Although the manufacture of PCBs was stopped over 30 years ago, they are still being detected in various environments (e.g., air, soil, dust, sediment, food) [1–6]. Because of the ubiquitous presence of PCBs in the environment, humans can be exposed to PCBs through several routes: inhalation of contaminated air (both outdoor and indoor); ingestion of contaminated food; and dermal contact with contaminated surfaces. Studies have showed that the dietary ingestion, e.g., through consumption of contaminated fish or oil, is the primary route of exposure to PCBs, and adverse health effects in both children and adults have been linked to PCB exposure [1]. PCBs have been found in building caulking materials from schools and other public buildings, posing widespread exposure hazards [7,8].

Toxicity equivalent factors (TEFs) were established for 12 coplanar PCBs [9]. Among them, the three non-ortho coplanar PCBs namely, PCB-77, PCB-126, and PCB-169 are structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The TEF values estimated for these coplanar PCBs are: 0.1 for PCB-126, 0.01 for PCB-169, and 0.0001 for PCB-77 [9]. The WHO re-evaluated the TEF values for PCDD/Fs in 2005 and decided to use half order of mag-

Abbreviations: Abs, antibodies; BSA, bovine serum albumin; DCM, dichloromethane; DDW, double-distilled water; ECD, electron capture detection; ELISAs, enzyme linked immunosorbent assays; GC/MS, gas chromatography/mass spectrometry; HpCDF, 1,2,3,4,6,7,8-heptachlorodibenzofuran; HRP, horseradish peroxidase; HxCDF, 2,3,4,6,7,8-hexachlorodibenzofuran; IAP, immuno-affinity purification; IgG, immunoglobulin; KLH, Keyhole limpet hemocyanin; MMT, Monitoring and Measurement Technology; MTMOS, methyltrimethoxysilane: ON, overnight; PBS, phosphate buffered saline; PBST, PBS containing 0.1% Tween-20; PCBs, polychlorinated biphenyls; RT, room temperature; SITE, Superfund Innovative Technology Evaluation; TCCD, tetrachlorodibenzo-*p*-dioxin; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TEFs, toxicity equivalent factors; TEQ, toxic equivalent; TMB, 3,3′,5,5′-tetramethyl benzidine; THEOS(, 2-hydroxyethyl)orthosilicate; TMOS, tetramethoxysilane.

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nitude increments on a logarithmic scale of 0.03, 0.1, 0.3, etc. [10]. The TEFs for PCB-126 and PCB-77 remain unchanged and PCB-169 was increased to 0.03. Concentrations of the three non-ortho PCBs in environmental samples were generally lower than those of other PCB congeners, but, nevertheless, their toxic equivalent (TEQ) values are not negligible and they cannot be overlooked. Multi-step cleanup procedures must be applied to soil and sediment specimens prior to analysis by gas chromatography/mass spectrometry (GC/MS) or GC with electron capture detection (ECD) [3–6]. These procedures usually involve acid wash and/or size exclusion, silica matrices, and/or Florisil column chromatography. Sample preparation and cleanup often form one of the most time-consuming steps in the overall analysis. Even when cleanup procedures are applied, the quantitative determination of trace levels of contaminants in complex sample matrices can be difficult. Thus, cost-effective methods are desired to reduce the overall costs and to improve monitoring efficiency particularly in large-scale studies.

Immunoaffinity purification (IAP) is one of the most powerful techniques for single-step isolation and purification of individual compounds or classes of compounds from liquid matrices [11–16]. The IAP technique has been widely applied for over four decades for pharmaceutical and biomedical trace analysis, and in the more recent decades for analysis of environmental contaminants and pesticide residues, in the fields of occupational and environmental health monitoring, forensic examinations, and food safety analysis [17]. The varied and complex matrices that serve as sources for analyte monitoring in soil and sediment samples, the low concentrations of the analytes within those samples, and the presence of compounds that interfere with the analytical process, raised the need for a highly specific, quick and cost-effective method for cleaning and concentrating the tested materials. In the past few years we have developed a novel IAP technology, based on the entrapment of antibodies (Abs) in a ceramic SiO_2 matrix termed sol–gel, which enables efficient, one-step cleanup and concentration of analytes from large-volume crude samples [17]. The technology was applied to entrapment of a wide variety of monoclonal, polyclonal and purified immunoglobulins (IgGs), and was proven to be highly efficient in terms of analyte recovery and reduction of matrix interference (for review see [17] and references therein and, [18–25]). Although the sol–gel-based IAP method was found to be highly effective, its application to environmental samples is still limited and its application to analytes of a variety of origins still needs to be evaluated and optimized.

Currently, high-resolution GC/high-resolution MS forms the widely accepted and reliable technique for quantification of PCB contaminants. However, although this technique is reliable and sensitive, it is time consuming, requires expensive equipment, and must be performed by highly trained personnel. Receptor gene assays, such as the chemical activated luciferase gene expression assay (CALUX, [26]) have recently been developed. This method, however, is problematic in that it employs maintenance of a cell culture, requires skilled personnel, elaborate equipment and a license to conduct the assay and, most important, it is not specific to a given compound. Immunoassays (e.g., enzyme linked immunosorbent assays, ELISAs) provide a good alternative as a highly sensitive, reproducible, rapid and cost-effective method.

Indeed, in the past two decades several immunoassays (ELISAs, surface plasmon resonance biosensors and commercial PCB test kits) have been developed and introduced to the market, and they are capable of detecting a wide variety of PCBs with detection limits at sub-microgram levels depending on the tested compound and the sample processing procedure [27–30]. Employment of these assays for environmental and food analysis often involves, as in the chemical instrumental analyses, tedious sample preparation procedures before the tests can be reliably applied. IAP can, thus, provide a good approach through a single-step cleanup that results in a sample that can be further analyzed by chemical–instrumental or immunochemical methods. To the best of our knowledge, no study so far has coupled both immunochemical methods, i.e., sol–gel-based IAP followed by ELISA, to determine recovery efficiency and to monitor PCBs recovered from environmental samples. Such a combination might simplify monitoring in a time- and cost-effective manner.

In the present paper we describe the development of two PCB ELISAs that use goat anti-PCB purified IgGs, and employment of the IgGs in the development of a sol–gel-based IAP method for cleanup of soil and sediment samples for determination of PCB-126. We also describe the further evaluation of the efficiency of a sol–gel-based IAP method in purifying PCB-126 from spiked soil/sediment samples and in eliminating matrix interferences to a degree that enables analysis of the purified samples by ELISA.

2. Materials and methods

2.1. Materials

PCB-77, PCB-118, PCB-126 and PCB-169 were purchased from Dr. Ehrenstorfer, GmbH, Augsburg, Germany. Aroclors (1232, 1248, 1260) were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA), and furans (hexachlorodibenzofuran 2,3,4,6,7,8-HxCDF and heptachlorodibenzofuran 1,2,3,4,6,7,8-HpCDF) were purchased from Cambridge Isotope Labs (Andover, MA, USA) and AccuStandard, Inc. (New Haven, CT, USA), respectively.

2.2. Immunochemical methods

2.2.1. Antibodies and conjugates

Polyclonal Abs 2504 were generated in a goat using a 3′,4,4′-trichloro-(3-thiobiphenyl) hapten linked to keyhole limpet hemocyanin (KLH) by means of 6-bromohexanoic acid. Rabbit anti-coplanar PCB, polyclonal Abs, and a coplanar PCB–horseradish peroxidase (HRP) conjugate were purchased from Abraxis LLC (Warminster, PA, USA).

PCB–BSA coating conjugate (Co-Ag 560-52) was generated using 4-(2,4,5-trichlorophenoxy)butyric acid conjugated to BSA [27]. Protein content of the conjugate was determined with Bradford reagent (Bio-Rad protein assay, Bio-Rad Laboratories, GmbH, Munich, Germany) from a BSA standard curve and was found to be $3\,mg\,mL^{-1}$.

2.2.2. Development of coplanar PCB competitive ELISA using anti-PCB goat IgG and a PCB–BSA coating conjugate (Format I ELISA)

2.2.2.1. Purification of anti-PCB IgGs from goat whole antiserum. A 2.5-mL aliquot of protein G, immobilized on cross-linked 4% agarose (Sigma) was packed in a Pasteur pipette at room temperature. The column volume obtained was 0.4 mL. The column was washed 4 times with 1 mL of 20 mM sodium phosphate buffer, pH 7.0 followed by an additional wash with 0.4 mL of 0.1 M glycine buffer, pH 2.7, and 4 more washes with 1 mL of 20 mM sodium phosphate buffer, pH 7.0. A 0.5-mL aliquot of anti-PCB goat antiserum 2504 was mixed with 4.5 mL of 20 mM sodium phosphate buffer, pH 7.0 and applied to the column, and the wash-through was collected and applied to the column twice more, to ensure maximal binding. The column was washed 10 times with 1 mL of 20 mM sodium phosphate buffer, pH 7.0, and elution of IgG was performed with 4 washes of 1 mL 0.1 M glycine buffer, pH 2.7. The glycine buffer fractions were collected into tubes containing 40 μL of 1 M Tris, pH 9.0, giving a mixture final pH of about 6.5 and the collected fractions, containing the purified IgGs, were combined and concentrated with Centricon Ultracel YM-30 tubes (Millipore,

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Billerica, MA, USA). The IgG fraction was brought to a final volume of 0.5 mL by addition of 10 mM NaH_2PO_4 and 150 mM NaCl, pH 7.2 (phosphate buffered saline, PBS) and stored in aliquots at 4 °C.

2.2.2.2. Search for optimal assay conditions: checkerboard experiments. Four serial dilutions, ranging from 1:500 to 1:4000 (equivalent to 600–75 ng 100 μL^{-1}), of PCB–BSA Co-Ag 560-52 coating conjugate (diluted in 0.5 M carbonate buffer, pH 9.6), were adsorbed, in duplicates, onto a 96-well microplate (Nunc MaxiSorp ELISA Plates, Roskilde, Denmark) and incubated overnight (ON). Additional wells, coated with an equivalent amount of bovine serum albumin (BSA, Albumin solution from bovine serum, RIA grade Fraction V, minimum 96%) (Sigma), served as a control to determine non-specific binding of the secondary Ab to the microplate, or the IgGs. Plates were washed 3 times with PBS containing 0.1% Tween-20 (PBST), and 5 serial dilutions of goat 2504 IgG (100 μL diluted 1:500–1:8000 in PBS) were then added to the plate and incubated ON at 4 °C. Wells with no IgG served to determine the non-specific binding of the reagents to the microplate wells. Plates were washed 3 times with PBST, and 100 μL of a secondary Ab (rabbit anti-goat HRP conjugate, affinity purified; Sigma), diluted 1:40,000 in PBST, were added to the plate and incubated at room temperature for 2 h. At the end of the incubation the plates were washed with PBST, 100 μL of a colorimetric 3,3′,5,5′-tetramethyl benzidine substrate (TMB, soluble colorimetric substrate) (Thermo Scientific, Rockford, IL, USA) was added, and the reaction was terminated by addition of 50 μL of 4 M H_2SO_4. The reaction was monitored with a colorimetric ELISA reader at 450 nm.

2.2.2.3. Calibration curves. PCB–BSA Co-Ag 560-52 coating conjugate (diluted 1:1000 in 0.5 M carbonate buffer, pH 9.6, equivalent to 0.3 μg 100 μL^{-1}) was adsorbed to microplates as above, and incubated overnight (ON) at 4 °C. Six wells were coated with an equivalent amount of BSA and served as a control to determine non-specific binding. Plates were washed 3 times with PBST, and 12 serial dilutions of PCB standards in PBS ranging from 0.0048 to 10 ng 50 μL^{-1} (PCB-77) or from 0.012 to 25 ng 50 μL^{-1} (PCB-126) were added in a volume of 50 μL, together with 50 μL of the purified IgG, which had been diluted 1:250 in PBS. Six wells received only PBS and served to determine maximal binding in the absence of PCB. Plates were incubated ON, washed 3 times with PBST as above, and 100 μL of a secondary Ab (rabbit anti-goat conjugated to HRP, diluted 1:40,000 in PBST) were added to the plate and incubated for 2 h at room temperature. At the end of the incubation the plates were washed with PBST, and 100 μL of a colorimetric TMB substrate (Dako, Glostrup, Denmark) was added to them. The reaction was stopped as above, and the signal was monitored with an ELISA reader at 450 nm.

2.2.2.4. Determination of cross reactivity. Cross reactivity was determined with the competitive ELISA as described in Section 2.2.2.3. Plates were coated with the Co-Ag 560-52 conjugate, incubated ON at 4 °C, washed 3 times with PBST, and 12 serial dilutions of the target analytes in PBS (ranging from 10 to 0.0048 ng 50 μL^{-1}) were added in a volume of 50 μL, together with 50 μL of goat 2504 IgG that had been diluted 1:250 in PBS. Six wells received only PBS and served to determine maximal binding in the absence of PCB. Plates were incubated ON at 4 °C, washed 3 times with PBST as above, and 100 μL of a secondary Ab (rabbit anti-goat conjugated to HRP, diluted 1:40,000 in PBST) was added, and the plates were incubated for 2 h at room temperature. At the end of the incubation the plates were washed with PBST, and 100 μL of a colorimetric TMB substrate were added as above. The reaction was monitored with an ELISA reader at 450 nm. The following compounds were tested for cross reactivity: PCBs (126, 169, 118), Aroclors (1232, 1248, 1260), and furans (hexachlorodibenzofuran 2,3,4,6,7,8-HxCDF and heptachlorodibenzofuran 1,2,3,4,6,7,8-HpCDF).

2.2.3. Coplanar PCB competitive ELISA using anti-PCB goat antiserum or purified IgGs and a PCB–HRP conjugate (Format II ELISA)

2.2.3.1. Calibration curve. ELISA white plates (Nunc MaxiSorp, ELISA White Plates, Roskilde, Denmark) were coated with donkey anti-goat Abs (Sigma, 1 μg 100 μL^{-1} made up in 0.5 M carbonate buffer, pH 9.6), in duplicates, and incubated ON at 4 °C. The plates were washed 3 times with PBST, and 100 μL of threefold-concentrated goat antiserum 2504 or of a 1:100 dilution of its purified IgG in PBS were added to the plates. Wells with PBS instead of the primary Abs served as a reaction background control. The plates were incubated ON at 4 °C, washed 3 times with PBST and 12 serial dilutions of PCB standard (PCB-126), diluted in PBS containing 10% methanol (PBS-M), ranging from 0.049 to 100 ng 50 μL^{-1}, were added to the plates. Assays were performed in a similar manner using IgGs except that the standard curve ranged from 0.0049 to 10 ng 50 μL^{-1}. Fifty-microliter aliquots of a 1:10,000 dilution of PCB–HRP conjugate (Abraxis, Los Angeles, CA, USA) in PBS containing 1% BSA were then added to all wells. Additional wells with no competing PCB, to which PBS-M was added, served to determine maximal binding (designated as 100%). The reaction was incubated for 90 min at room temperature (RT), the plates were washed 3 times with PBST, and 100 μL of a chemiluminescent TMB substrate (SuperSignal ELISA Pico Chemiluminescent Substrate, Thermo Fisher Scientific, Rockford, IL, USA) were added. The reaction was monitored with a chemiluminescent ELISA reader.

2.2.3.2. Determination of cross reactivity. Cross reactivity was determined with the competitive Format II ELISA as described in Section 2.2.3.1. Plates were coated with the donkey anti-goat Abs, incubated ON at 4 °C and washed 3 times with PBST. The threefold-concentrated goat antiserum 2504, diluted 1:100 in PBS, was then added to the plates and incubated as above. Fifty-microliter aliquots of 12 serial dilutions of the tested compounds in PBS-M (ranging from 0.049 to 100 ng 50 μL^{-1}) were added, together with 50 μL of a 1:10,000 dilution of PCB–HRP in PBS containing 1% BSA. Six wells received only PBS-M and served to determine maximal binding in the absence of PCB. The reaction was incubated for 90 min at RT, the plates were washed 3 times with PBST, and 100 μL of a chemiluminescent TMB substrate were added. The reaction was monitored with a chemiluminescent ELISA reader.

2.2.4. Coplanar PCB competitive ELISA using anti-PCB rabbit antiserum and a PCB–HRP conjugate (Format III ELISA)

2.2.4.1. Calibration curve and determination of cross reactivity. ELISA White Plates (Nunc MaxiSorp, ELISA White Plates, Roskilde, Denmark), were coated, in duplicates, with protein A, derived from *S. aureus* (Sigma), made up in 0.5 M carbonate buffer, pH 9.6, at 1 μg 150 μL^{-1}, and were incubated ON at 4 °C. The plates were washed 3 times with PBST, and 50-μL aliquots of PCB standards (PCB-126) serially diluted in PBS-M to concentrations ranging from 0.0005 to 1 ng 50 μL^{-1} were added. This was followed by the addition of 50 μL aliquots of anti-PCB rabbit antiserum (Abraxis, Los Angeles, CA, USA) diluted 1:4000 in PBS containing 1% BSA (PBS–BSA). Wells with no competing PCB served to determine maximal binding (designated as 100%). Wells with PBS–BSA instead of the antiserum served as a background control. Plates were incubated for 30 min at RT, and 50-μL aliquots of PCB–HRP conjugate (Abraxis, Los Angeles, CA), diluted 1:10,000 in PBS containing 0.5% PBS-BSA, were added to all wells. The plates were incubated for an additional 90 min at RT and washed 3 times with PBST, and 150 μL of SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Rockford, IL, USA) was added and the reaction

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monitored as in Section 2.2.3.2. Cross reactivity was determined under identical experimental conditions.

2.3. Sol–gel-based immunoaffinity cleanup

2.3.1. Column preparation

IgG entrapment was carried out by a two-step procedure in which hydrolysis was followed by polymerization of tetramethoxysilane (TMOS) (Aldrich, 99%, Karlsruhe, Germany) as previously described [31]. Briefly, an acidic silica sol solution was obtained by mixing TMOS with 2.5 mM HCl in double-distilled water (DDW) at molar ratios of 1:4, 1:6, 1:8 and 1:12. The mixture was stirred for 1 min until a clear solution was obtained, and was then sonicated for 30 min in an ELMA ultrasonicator bath (model T-460/H, 285 W, 2.75 l Singen-Hohentwiel, Germany). The reaction was performed in a well-ventilated fume hood. Sixty-microliter aliquots of anti-PCB goat 2504 IgGs were premixed with 316-, 380-, 440- and 564-μL of 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES, 99.99%) (Sigma), pH 7.6, and the mixtures were added to 376-, 440-, 500- and 624-μL of the pre-hydrolyzed 1:4, 1:6, 1:8 and 1:12 TMOS mixtures, respectively. Control columns, which did not contain IgGs (referred to below as 'empty') were prepared in a similar manner, except that the HEPES was mixed with an equivalent volume of pre-hydrolyzed TMOS at each molar ratio, as indicated above. The solution was mixed quickly for 5 s, and gelation occurred within 1–2 min. After 30 min the gels, in total volumes of 0.752, 0.880, 1.0 and 1.248 mL, were washed with 2 mL of HEPES buffer, pH 7.6, and stored at 4 °C with 2 mL of HEPES buffer pH 7.6, on top. Best results were obtained with gels that had been stored ON at 4 °C and used on the second day after preparation. The gels exhibited high stability and could be used for over 2 months after preparation.

2.3.2. Sample loading and elution of PCB-126 from sol–gel IAP columns

Wet gels were thoroughly crushed and were transferred into Econo-Pac disposable chromatography columns (Bio-Rad, Philadelphia, PA, USA). The resulting sol–gel columns were washed with 50 mL of PBS prior to sample application. For optimal binding, the columns were kept under buffer at all times during the experiment. PCB standard (PCB-126, 400 ng), unspiked soil/sediment samples or spiked soil/sediment samples (prepared as described in Section 2.3.3) spiked with 400 ng of PCB-126, were applied, in 1 mL of PBS, to the doped or 'empty' sol–gel columns. Unbound PCB was removed by washing with 20 mL of DDW. Elution was performed with 10 mL of absolute ethanol (PESTI-S, Bio-Lab, Jerusalem, Israel), unless otherwise indicated PCB content was determined by ELISA (ELISA III, as described in Section 2.2.4), except that the 50% methanol in the reaction buffer was replaced with 50% ethanol (to comply with the solvent of the samples eluted form the sol–gel columns). Samples were diluted 1:2 in 2× PBS prior to analysis, to achieve a final ethanol concentration of 50%. Higher dilutions, if required, were carried out in PBS-E.

2.3.3. Preparation of spiked and unspiked soil/sediment samples

Soil/sediment samples (N = 9) were collected from five contaminated sites in a field study conducted under an EPA Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) program [8,9,32,33]. The soil and sediment samples were extracted as follows: 10 mL of dichloromethane (DCM) (PESTI-S, Bio-Lab, Jerusalem, Israel) was added to 2 g of sample, which was then sonicated for 15 min at RT. The supernatant was removed and passed through a quartz fiber filter (Millipore, Bedford, MA, USA) that was wetted with DCM prior to use. The procedure was repeated once more, followed by a single wash of the sample with 5 mL of DCM. The filtrates, totaling 25 mL, were combined and each sample was divided into two 12.5-mL portions, each corresponding to 1 g of sample, evaporated to dryness under a slow stream of N_2 at room temperature and stored at −20 °C pending use.

2.3.4. Analysis of soil/sediment samples by ELISA

The unspiked samples were dissolved in 1 mL of PBS-M, and the ones to be spiked in 1 mL of PBS-M containing 5.12 ng of PCB-126. The unspiked samples were tested for matrix interference, i.e., the degree of binding interference of the PCB–HRP conjugate to the antiserum adsorbed to the plate, in the absence of competing PCB. The ability of the assay to accurately determine the amount of PCB was tested with the spiked samples. The samples – undiluted, and diluted with PBS-M at 1:2, 1:4, 1:8 and 1:16 (equivalent to 50–3.125 mg soil/sediment) were subjected to Format II or Format III ELISA as described above (Sections 2.2.3 and 2.2.4, respectively).

For IAP analysis, unspiked and spiked samples were reconstituted in 1 mL of PBS alone or in PBS containing 400 ng of PCB-126, respectively, and each sample was applied to an 'empty' and to an IgG-containing sol–gel column. Sol–gel eluates diluted with PBS-E at 1:10, 1:20, 1:40, 1:80 and 1:160 (equivalent to 2.5–0.15 mg purified extract) were subjected to Format III ELISA as described above (Section 2.2.4).

2.4. Data transformation

PCB concentration was calculated by ELISA from a calibration curve after linearization of the data by transformation to a logit–log plot by means of Microcal Origin software, Version 6.0 (Microcal Software, Northampton, MA, USA). All samples were tested in duplicate, in four or five dilutions that were within the range of the standard curve. Slopes of all samples were tested for parallelism with the standard curve, by testing for homogeneity of regression slopes according to Sokal and Rohlf [34]. Only samples whose regression lines were parallel to the standard curve were considered.

3. Results

3.1. Development of a PCB ELISA using purified goat anti-PCB IgGs

The first part of the study focused on development of an indirect competitive ELISA in which the coating antigen (PCB–BSA) conjugate was adsorbed to the microplate and competed with the free PCB present in a standard solution or sample, on binding to an Ab added to the reaction mixture. In order to establish the assay it was necessary to find the optimal working conditions, i.e., the amounts of the coating Ag-conjugate and of the antiserum for which the signal to noise ratio was the highest. Examination of a wide range of dilutions of coating conjugate and antiserum, in the presence and absence of a variety of blockers (normal rabbit serum, ovalbumin and milk powder), by means of checkerboard experiments, revealed very high non-specific binding and lack of dose dependency, and the results did not support development of an immunoassay. An ELISA could be established only when IgGs were purified from the goat antiserum. Purification of IgGs, by means of a protein G affinity column, resulted in a dose-dependent binding activity of the IgG to the coating antigen (Fig. 1), with a low non-specific binding to the carrier protein.

Attempts to establish a calibration curve with PCB-77 and PCB-126 using the purified IgGs revealed (Fig. 2) that the IgGs exhibited the highest affinity toward PCB-77 (I_{50} value of 24 ± 7 ng mL^{-1}) and the assay showed a detection limit (I_{20}) of 6 ± 2 ng mL^{-1} (Table 1). Interestingly, PCB-77 was unable to fully compete with the coated hapten-conjugate adsorbed to the plate; it reached a maximal "competition value" of 60% at 2.5 ng 50 μL^{-1} and further increase in

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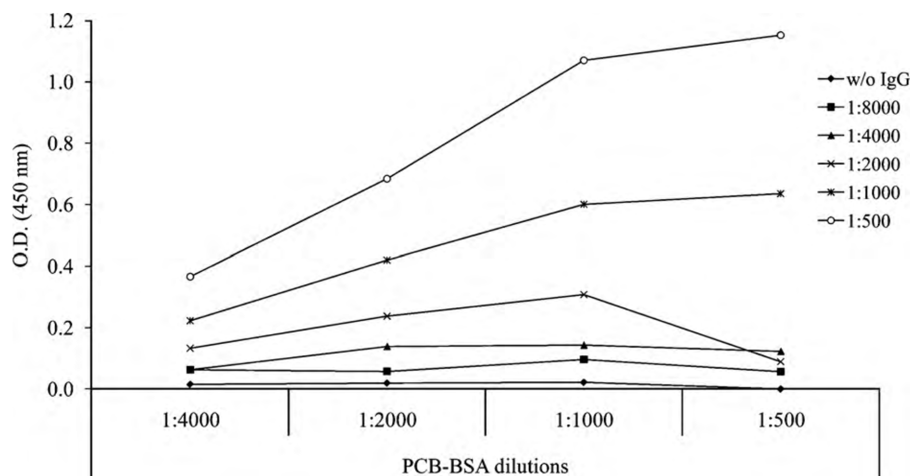

Fig. 1. Dose response of binding of anti-PCB goat IgG (2504) to PCB–BSA conjugate adsorbed to a 96-well microplate. Experimental details are as for Format I ELISA checkerboard experiments described in Section 2.2.2.2. Data represent net binding, namely, binding of IgGs to the coating conjugate after subtraction of the non-specific binding, i.e., binding in the presence of BSA instead of the coating PCB–BSA antigen.

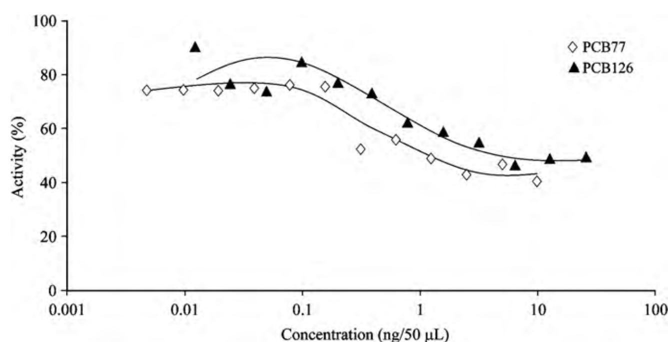

Fig. 2. Representative standard curve of PCB-77 and PCB-126 ELISA obtained with anti-PCB goat 2504 IgGs. Experimental details are as for Format I ELISA calibration curve described in Section 2.2.2.3.

the amount of PCB did not reduce binding of the IgG to the adsorbed conjugate. It is possible that the IgG preparation contained a mixed population of IgGs, all of which recognized the hapten–BSA conjugate and bound to it on the plate, but only part (60%) of which recognized PCB-77 whereas the other IgGs were unaffected and therefore continued to bind to the adsorbed conjugate in the presence of increasing amounts of PCB-77. The IgG also recognized PCB-126, although at a lower affinity (100 ± 1 ng mL^{-1}). PCB-169 was not recognized by the IgG (Table 1).

Comparison of the I_{50} and limit of detection of the purified IgGs in Format I ELISA with those obtained in a different ELISA format, i.e., Format II in which the tested PCB competes with a PCB–HRP conjugate on binding to IgGs adsorbed to the microplate, revealed a slightly lower affinity and a comparable limit of detection for PCB-77 (42 ± 2 and 11 ± 1 ng mL^{-1}, respectively) and a comparable value for PCB-126 (110 ± 10 and 26 ± 2 ng mL^{-1}, respectively) (Table 1). The affinity of the IgGs toward PCB-77 in this format was also higher than that toward PCB-126 (Table 1). Despite our inability to develop an assay with the whole antiserum in Format I ELISA, we were able to develop an assay with the antiserum in Format II. The affinity and limit of detection of the whole antiserum did not differ markedly from those of the purified IgG for PCB-77 (54 ± 3 and 9 ± 2 ng mL^{-1}, respectively) but exhibited higher values for PCB-126 (180 ± 29 and 63 ± 27 ng mL^{-1}, respectively) (Table 1).

The I_{50} and I_{20} values were also compared with those obtained with a commercial antiserum generated in rabbits (Format III ELISA). As indicated in Table 1 the data revealed similar values (affinity and limit of detection of 26 ± 6 and 4 ± 0.9 ng mL^{-1}, respectively) to those obtained with the goat antiserum or purified IgGs for PCB-77. However, the affinity of the rabbit antiserum in this format toward PCB-126 was higher by two orders of magnitude and the detection limit – significantly lower (0.98 ± 0.13 and 0.15 ± 0.04 ng mL^{-1}, respectively) (Table 1). PCB-169, which was not even recognized by the goat antiserum or purified IgG, also exhibited high affinity (0.29 ± 0.03 ng mL^{-1}) and its detection

Table 1

I_{50} and I_{20} values of goat anti-PCB 2504 antiserum, goat 2504 anti-PCB-purified IgGs and rabbit anti-PCB antiserum in various ELISA formats.

Coating conjugate	Goat anti-PCB 2504 IgG		Goat anti-PCB 2504 antiserum		Rabbit anti-PCB antiserum	
PCB–BSA	I_{50} (ng mL^{-1})[a]	I_{20} (ng mL^{-1})[a]				
PCB-77	24 ± 7 ($n = 3$)	6 ± 2 ($n = 5$)	n.d.	n.d.	n.t.	n.t.
PCB-126	100 ± 1 ($n = 2$)	22 ± 18 ($n = 2$)	n.d.	n.d.	n.t.	n.t.
PCB-169	>200	>200	n.d.	n.d.	n.t.	n.t.
Coating conjugate	Goat anti-PCB 2504 IgG		Goat anti-PCB 2504 antiserum		Rabbit anti-PCB antiserum	
PCB–HRP	I_{50} (ng mL^{-1})[b]	I_{20} (ng mL^{-1})[b]	I_{50} (ng mL^{-1})[b]	I_{20} (ng mL^{-1})[b]	I_{50} (ng mL^{-1})[c]	I_{20} (ng mL^{-1})[c]
PCB-77	42 ± 2 ($n = 2$)	11 ± 1 ($n = 2$)	54 ± 3 ($n = 3$)	9 ± 2 ($n = 3$)	26 ± 6 ($n = 2$)	4 ± 0.9 ($n = 2$)
PCB-126	110 ± 10 ($n = 2$)	26 ± 2 ($n = 2$)	180 ± 29 ($n = 4$)	63 ± 27 ($n = 3$)	0.98 ± 0.13 ($n = 5$)	0.15 ± 0.04 ($n = 5$)
PCB-169	>200	>200	>2000	>2000	0.29 ± 0.03 ($n = 5$)	0.10 ± 0.03 ($n = 5$)

n.t.: not tested; n.d.: could not be detected.

[a] Values were determined from calibration curves by Format I ELISA.

[b] Values were determined from calibration curves by Format II ELISA.

[c] Values were determined from calibration curves by Format III ELISA.

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Table 2
Cross reactivity of goat anti-PCB 2504 antiserum, goat anti-PCB purified IgGs, and rabbit anti-PCB antiserum with various PCBs, Aroclors and furans.

Compound	Cross reactivity		
	Goat-2504 IgG PCB–BSA conjugate[a]	Goat-2504 antiserum PCB–HRP conjugate[b]	Rabbit antiserum PCB–HRP conjugate[c]
PCB-77	100	280	3
PCB-126	19	100	100
PCB-169	0	0	164
PCB-118	0	n.t.	0
Aroclor 1232	0	0	0
Aroclor 1248	0	n.t.	0
Aroclor 1260	0	0	0
2,3,4,6,7,8-HxCDF	0	0	0
1,2,3,4,6,7,8-HpCDF	100	0	0

Cross reactivity represents the ratio (expressed as a percentage) between the concentration of PCB-77 that caused a 50% decrease in binding of the IgG to the coating antigen adsorbed onto the microplate and the concentration of tested compound that caused the same inhibition (Format I ELISA); or between the concentration of PCB-126 that caused a 50% decrease in binding of PCB-HRP conjugate to the antiserum adsorbed onto the microplate and the concentration of the tested compound that caused the same inhibition (ELISA Formats II and III). n.t.: not tested.

[a] Values were determined from calibration curves by Format I ELISA.
[b] Values were determined from calibration curves by Format II ELISA.
[c] Values were determined from calibration curves by Format III ELISA.

limit was the lowest (0.10 ± 0.03 ng mL^{-1}). Examination of all of the above values revealed that goat anti-PCB IgGs exhibited the highest affinity and lowest limit of detection toward PCB-77, comparable with those of the commercial anti-rabbit Ab. The latter antiserum exhibited the highest affinity and lowest limit of detection toward PCB-169. The rabbit antiserum was the only one that recognized PCB-169. No other tested antiserum or purified IgG preparation showed any cross reactivity with this compound in any of the tested formats.

Further examination of the ability of the purified goat IgG or of the whole antiserum to cross react with PCB-118, 1,2,3,4,6,7,8-HpCDF, and 2,3,4,6,7,8-HxCDF revealed no cross reactivity with PCB-118 and 2,3,4,6,7,8-HxCDF, but 100% cross reactivity with 1,2,3,4,6,7,8-HpCDF (Table 2). In regards to coplanar PCB composition, the three Aroclors tested (1232, 1248, 1260) contain minute amounts of PCB 169. No reaction was detected due to the selectivity of the antibody and the extremely low amount (<0.0001%) of PCB 169 in the Aroclors.

3.2. *Analysis of spiked and unspiked soil/sediment samples by ELISA*

Soil samples were extracted, spiked with PCB-126 at 5.12 ng mL^{-1}, and tested for the analyte content with Format III ELISA. As can be seen in Table 3, PCB-126 could be detected in only 3 out of the 8 soil samples, and the amounts detected in those samples represented only 12–31% of the spiked amount. Analysis of most of the samples resulted in curves that did not parallel the calibration curve (designated as n.d. in Table 3). Unspiked soil samples were tested for possible matrix interference and for any binding to the specific antibody, HRP-PCB conjugate or an inactivation of the HRP enzyme. The results revealed interference of 35–85% in the presence of 3.125–50 mg soil, respectively (data not shown). Similar data were obtained in Format II ELISA.

Table 3
Determination of PCB-126 in spiked soil samples by ELISA.

Sample	PCB-126 determined (ng mL^{-1})	Recovery (%)
Buffer	5.12	100
Soil #1	0.832	16
Soil #2	0.608	12
Soil #3	1.60	31
Soil #4	n.d.	n.d.
Soil #5	n.d.	n.d.
Soil #6	n.d.	n.d.
Soil #7	n.d.	n.d.
Soil #8	n.d.	n.d.

Soil samples were spiked with 5.12 ng PCB-126. Soil samples (undiluted, diluted 1:2, 1:4, 1:8 and 1:16, representing an equivalent of 50–3.125 mg of soil) were tested by Format III ELISA. Recovery represents the ratio (as a percentage) between the amount of PCB-126 recovered from spiked soil samples and that recovered from spiked buffer (designated as 100%). Values were obtained after logit–log transformation of the data by means of Microcal Origin software. n.d.: not detected.

3.3. *Sol–gel-based IAP of PCB-126*

Four sol–gel formats were generated, with differing TMOS:HCl ratios (1:4, 1:6, 1:8 and 1:12), and the binding capacity of the entrapped IgGs (60 μL of each) was examined by binding and eluting 400 ng of PCB-126. Elution was carried out with ethanol in one 10-mL fraction and the PCB content was determined with Format III ELISA. As indicated in Fig. 3, the highest activity was obtained with a 1:12 TMOS:HCl ratio which exhibited a binding capacity of 188 ng. The other three formats, with ratios of 1:4, 1:6 and 1:8 exhibited significantly lower binding capacities, of 45, 22, and 52 ng, respectively. The non-specific binding values of all the tested samples were high (241, 256, 305 and 200 ng for ratios of 1:4, 1:6, 1:8 and 1:12, respectively), and no significant differences could be detected between the various sol–gel formats. The best ratio of specific to non-specific binding was obtained at a TMOS:HCl ratio of 1:12, where the non-specific binding was the lowest and the total binding the highest.

Next, the elution profile of PCB-126 from the sol–gel IAP columns was evaluated. Sol–gel columns, with a TMOS:HCl ratio of 1:12, were prepared, with and without IgGs (60 μL); 400 ng of PCB-126 were applied to each of the columns, as above, and the compound was eluted in 10 fractions, each of 1 mL, instead of a single pooled fraction of 10 mL. Each fraction was tested for its PCB-126 content by means of Format III ELISA and a PCB-126 calibration curve. As can be seen in Fig. 4, 89% of the eluted analyte (231 ng out of a total recovery of 257 ng) was recovered in the first two fractions from the doped column, and all the other fractions had a residual PCB content. The PCB that bound non-specifically to the sol–gel polymer in the 'empty' columns, designated as background, eluted in the first four fractions (#1–4) and amounted to 79% of the recovered analyte, i.e., 93 ng out of a total recovery of 118 ng. The amount of PCB that bound non-specifically to the sol–gel in fractions #1 and #2 was 43 ng, and exhibited only 36% of the amount of PCB eluted from the doped column.

Next, the ability of the sol–gel IAP columns to effectively cleanup real samples prior to the detection of PCB 126 was tested. For that

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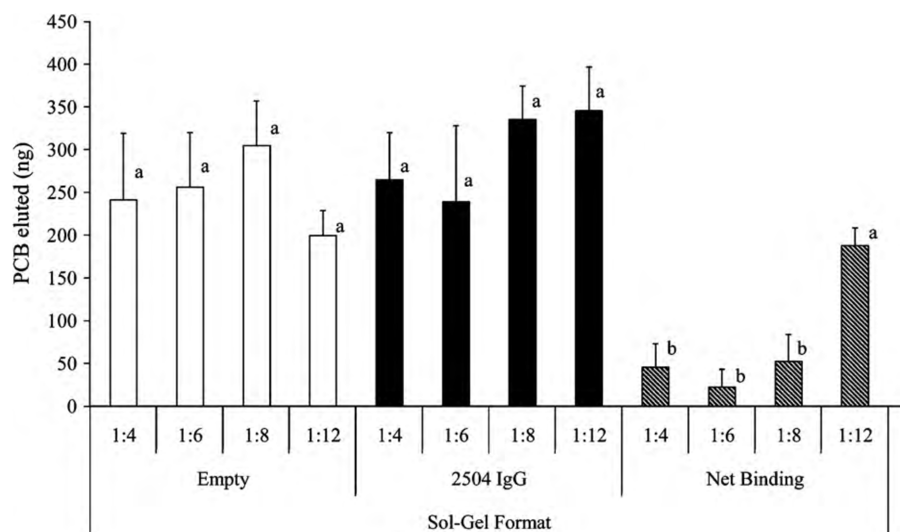

Fig. 3. Effects of various sol–gel formats (based on different TMOS:HCl ratios) on the activity of anti-PCB-entrapped 2504 IgGs. Amount of eluted PCB was determined by Format III ELISA from a PCB-126 standard curve. All samples were tested at 5 serial dilutions ranging from 1:8 to 1:128. Bars labeled 'empty' represent columns that were not doped with IgGs, these bars represent the non-specific binding. Bars labeled '2504 IgG' represent the total binding capacity and bars labeled 'net' represent the specific binding values (difference between the total binding and the binding of the 'empty' columns). Each bar represents the mean ± S.E.M. of 3–5 measurements. Statistical analysis was applied to each group (i.e., 'empty' '2504 IgG' and 'net') separately. Means with the same letter do not differ significantly at $p < 0.05$.

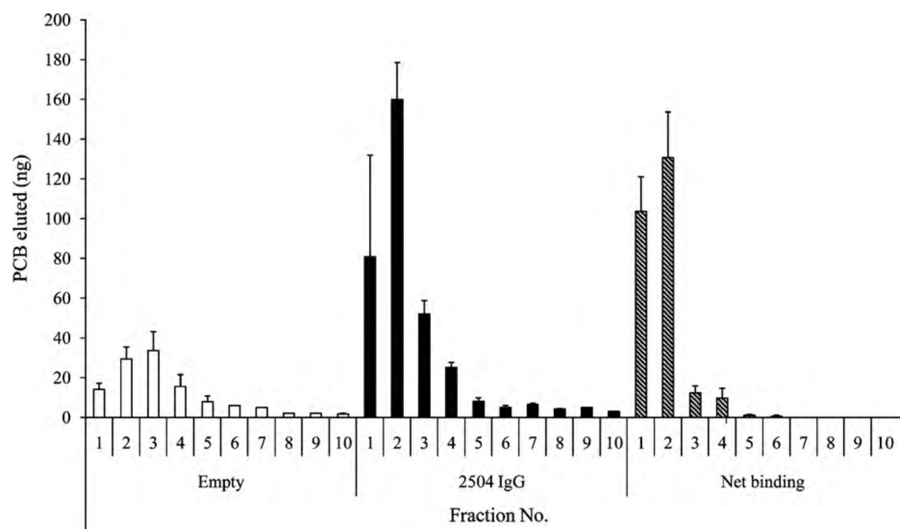

Fig. 4. Elution profile of PCB-126 from 'empty' and IgG-containing sol–gel columns. PCB-126 (400 ng) was loaded on a sol–gel column prepared at a TMOS:HCl ratio of 1:12 and doped with 60 μL of goat anti-PCB IgGs. PCB-126 was eluted in 10 fractions, each of 1 mL. All other experimental details are as described in the legend to Fig. 3. Fractions 1–5 were tested at 5 serial dilutions ranging from 1:8 to 1:128. Fractions 6–10 were tested at 5 serial dilutions ranging from 1:2 to 1:32. Each bar represents the mean ± S.E.M. of 3–5 measurements.

purpose 1 g of a contaminated soil sample (TR-1) was extracted [33]. The extract was spiked with 400 ng of PCB-126 and applied on sol–gel columns that contained 60 μL of goat anti-PCB IgGs. Elution was carried out with ethanol, and the amount of PCB recovered was determined with Format III ELISA and a PCB-126 calibration curve. Soil extracts contained an equivalent of 1.5–2.5 mg of cleaned extract. The data in Table 4 indicate a high recovery of PCB-126 from the soil/sediment extract No. 9. Unspiked soil/sediment samples that were tested in parallel did not contain any detectable amounts of PCB-126 (Table 4).

Table 4
PCB-126 recovery from a spiked soil/sediment sample by IgG doped sol–gel IAP columns.

Sample	PCB-126 (ng)
PCB-126 in buffer	112 ± 3 (n = 3)
PCB-126 in soil #9	216 ± 45 (n = 4)
Soil #9 un-spiked	<0.0075

The soil sample was spiked with 400 ng of PCB-126 and applied on sol–gel columns that contained 60 μL of goat anti-PCB IgGs. The analyte was eluted with 2 mL of absolute EtOH. Amount of recovered PCB was determined by Format III ELISA from a PCB-126 standard curve. All samples were tested at 5 serial dilutions, ranging from 1:10 to 1:160 (equivalent of 2.5–0.15 mg of purified extract). Data are presented as specific binding (binding to doped minus binding to 'empty' columns). Data represents the mean ± S.E.M. of 3 or 4 measurements.

4. Discussion

In the present study two ELISAs and a sol–gel-based IAP method for cleanup and monitoring of PCBs were developed. Both methods employed goat 2504 anti-PCB IgGs purified from an antiserum that was generated using 3′,4,4′-trichloro-(3-thiobiphenyl) hapten linked to KLH by means of 6-bromohexanoic acid. Two competitive ELISA formats were developed: an indirect competitive ELISA (designated as Format I ELISA) in which the coating hapten(Ag)–BSA conjugate was adsorbed to the microplate and competed with the

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free PCB present in a standard solution or a sample, on binding to the IgG added to the reaction mixture; and an assay (designated as Format II ELISA) in which the IgGs were adsorbed to the microplate wells via a secondary Ab, and the tested PCBs competed with a PCB–HRP conjugate on binding to IgGs adsorbed to the microplate. Interestingly, we were unable to develop a Format I ELISA with the whole antiserum, and the assay could be established only after IgGs have been purified from the antiserum by means of protein G. Once the IgGs have been purified we obtained a, dose-dependent binding activity of the IgG to the coating antigen (Fig. 1), with a low non-specific binding to the carrier protein. Employment of the purified IgGs in this ELISA format revealed (Fig. 2) that the IgGs exhibited the highest affinity toward PCB-77 (I_{50} value of 24 ± 7 ng mL^{-1}) and a detection limit (I_{20}) of 6 ± 2 ng mL^{-1} (Table 1). The reason for our inability to develop an ELISA with the whole antiserum is not yet clear. It may very well be that the titer of the IgGs that recognizes PCB-77 or PCB-126 is low relative to that of the IgGs that recognize PCB coupled to a carrier protein, e.g., the coating conjugate, and that the activity of this small fraction is masked in the presence of the proteins present in the whole unpurified serum.

The whole antiserum and the IgGs were also used to develop another ELISA format (Format II). In this format both preparations worked well, exhibiting similar affinities toward PCB-77 and PCB-126. The affinity of the IgG toward PCB-77 was also similar to that obtained with a commercial rabbit polyclonal PCB antiserum, but its affinity toward PCB-126 was much lower than that of the rabbit Ab (Table 1). The goat IgG was the only Ab that recognized the furan 1,2,3,4,6,7,8-HpCDF, which indicated a unique ability to recognize this compound. The Ab did not cross react with any other tested PCB, Aroclor or furan (Table 2). Another selective immunoassay (for PCB-169) that emerged from this study was based on the use of the rabbit antiserum in Format III ELISA. Availability of selective assays for individual compounds represents a major advantage in immunochemical analysis as it enables results obtained by means of immunoassays to be verified.

A wide variety of immunoassays have been developed in many laboratories based on monoclonal or polyclonal (rabbit or sheep) Abs. They comprise: ELISAs in various formats [27,35–37]; commercial kits [38–40]; a radioimmunoassay [41]; surface plasmon resonance biosensors [28]; and bioelectrochemical assays based on magnetic beads [29]. Most of the above assays were developed for Aroclors [27,36,37], one was developed for tetrachlorodibenzo-*p*-dioxin (TCCD) [35], and one for PCB-118 [28]. The assays reported in the present paper are the first to be developed for PCB-77, PCB-126 and PCB-169, in an antigen coating format. A commercial kit for those compounds (in an Ab coating format) is available from Abraxis LLC (Warminster, PA, USA). The cross reactivity with the furan enables development of an immunoassay for this compound and monitoring of its presence in real samples. Comparison of the limits of detection of all the ELISAs developed with those in the present study revealed values (0.1–63 ng mL^{-1}, Table 1) similar to or even lower than those reported for other immunoassays [35]. In general sub nanogram per milliliter detection limits (0.05–0.1 ng g^{-1}) for coplanar PCBs in soil (sample size = 10–50 g) can be achieved by conventional gas chromatography/high-resolution mass spectrometry (GC/HRMS) analysis [42,43]. However, multi-step cleanup procedures are required prior to GC/HRMS analysis.

The ability of the goat anti-PCB IgGs to recognize various coplanar PCBs, and the availability of a variety of immunoassay formats stimulated an attempt to develop an IAP method for PCBs, based on the sol–gel approach and combine it with ELISA analysis. The results clearly indicate that sol–gel-entrapped IgGs can bind PCB-126 and that the latter can easily be eluted by ethanol (Fig. 3). Previous studies have shown that changing a sol–gel format affects the physical properties of the sol–gel matrix, by changing its porosity, which, in turn, might affect the conformation and activity of the encapsulated biomolecules. Our attempts to determine the effects of various sol–gel formats on the activity of the entrapped IgGs revealed that, indeed, different formats exhibit different binding capacities. Thus, whereas a TMOS:HCl molar ratio of 1:12 resulted in a high binding capacity, ratios of 1:4, 1:6 and 1:8 were much less efficient, with values 4–8 times lower than those obtained with the 1:12 format (Fig. 3). In spite of our success in binding and eluting the PCB from the sol–gel IAP columns, the non-specific binding of the compound to the sol–gel matrix was very high: 50–75% of the amount applied to the column. Further optimization should be implemented in order to obtain higher recoveries. Possible measures include: use of different formats based on different monomers to which the PCBs might adhere with lower affinities, e.g., Tetrakis (2-hydroxyethyl)orthosilicate (THEOS) or methyltrimethoxysilane (M-TMOS); addition of different additives such as sugars, unrelated proteins, etc., that might minimize the non-specific binding sites in the matrix; use of different washing buffers or solvents; and use of differential elution conditions. It could also very well be that other PCBs, such as PCB-77, PCB-169 and the heptachlorodibenzofuran 1,2,3,4,6,7,8-HpCDF, that are recognized by the IgG or the whole goat or rabbit antiserum might behave differently on the sol–gel column; these should be tested as well. Previous sol–gel-based IAP studies carried out with a variety of compounds (some of which were lipophilic; tri-nitro-phenol [31], atrazine [44], and the pyrethroid bioallethrin [20]) revealed that careful optimization of the method can result in a very low non-specific binding (less than 20%). In spite of the relatively low recovery of the IAP method it should be noted that the obtained compound is highly pure and can be tested without any further purification by means of GC–MS or immunoassays as proven with the pyrethroid bioallethrin [20].

Evaluation of the minimal volume required for analyte elution revealed that nearly 90% of the analyte eluted in the first two fractions (Fig. 4). PCB-126, which bound non-specifically to the sol–gel itself, exhibited a slightly different elution pattern that spread over an additional two fractions. The differences in elution profiles enabled collection of just the first two fractions, which resulted in a significant decrease in the background as compared with that obtained with a 10-mL elution. In previous studies we found that efficient elution from sol–gel IAP columns was obtained with 10–20 mL of organic solvent [44,45]. This resulted in a ten-fold dilution of the eluted analyte with an organic solvent that was not tolerated by the ELISA and that had to be removed by evaporation. The small elution volume for PCB-126 that we achieved in the present study thus represents a major advantage: there is not much dilution of the sample, and there is no longer any need for an additional step, e.g., evaporation or solid-phase extraction, prior to quantitative analysis of the eluted sample.

The availability of a variety of PCB ELISAs with detection limits low enough to quantify PCBs in soil samples at the PCB levels of 50 μg g^{-1} or more that constitute soil contamination [37,40] enabled us to examine soil/sediment samples for occurrence of the PCBs recognized by the above antisera or IgGs, i.e., PCB-77 and PCB-126. Attempts to detect PCBs (specifically, PCB-126) in spiked soil/sediment samples (No. 1-8) revealed high matrix interference even in the presence of very small amounts of the tested matrix (3.12 mg soil extracts) in a manner that hampered the ability to quantify the analyte. In most cases the curves did not parallel the calibration curve, which impaired determination of the amount of the analyte in the sample, and in the few samples in which quantification could be achieved the detected amounts represented only 20–30% of the spiked analyte (Table 3). Immunoaffinity cleanup of the samples prior to the ELISA analysis completely eliminated matrix interference and enabled us to examine PCB-126 content in the purified extracts (equivalent of 2.5 mg of spiked soil/sediment extract) (Table 4), clearly indicating a good efficiency of the sol–gel

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IAP in purifying PCB. It is important to note that eluates were diluted by a factor of 10–160 prior to the ELISA, in order to bring the analyte concentrations within the range of the calibration curve. Lower dilutions were not tested and it may very well be that even at a 1:2 dilution (equivalent to 2.5 mg of spiked soil/sediment extracts) they would not have interfered with the assay.

Interestingly, the amount of PCB-126 recovered from the sol–gel IAP column when samples were applied in buffer was somewhat lower than that recovered from the spiked soil/sediment samples. The reason for this was not clear; it may very well be that this represents fluctuations between experiments, or that the sample components lowered the non-specific binding of the analyte to the sol–gel and thereby caused higher recovery. It should be noted that the reported amount of soil tested (equivalent to 1 g of soil/sediment extract) was the largest tested in the present study, and it could very well be that the sol–gel IAP method is capable of purifying much larger amounts under the same protocol. Further experiments, with larger amounts of soil/sediment extracts, spiked with PCB-126 and other PCBs that cross react with the IgGs, will have to be analyzed before a final evaluation of the recovery can be drawn.

Studies of PCB monitoring in soil by means of ELISA have been performed by several laboratories that used unspiked and spiked soil and sediment samples with a variety of analytes (Aroclor 1248, 1242, and the dioxin TCDD) [27,35,36,39,46,47]. Because of the differences between the extraction methods, pre-analysis treatments, sample types, and difficulties in determining the sample equivalent quantities that were tested in each assay, it is almost impossible to make comparisons between the various studies. In general, the results of these studies revealed that although ELISA can be used as a rapid screening method, the matrix does interfere with the assay, and the extraction method has a significant effect on the quality of the ELISA results. The general conclusion that emerged from these studies is that accurate quantitative determination of PCB quantities can be obtained only after a pre-analysis cleanup step [35,46] or dilution of the sample [35,36,46]. Post-extraction cleanup steps are also necessary for the determination of lipophilic dioxins in complex sample media such as sediment or soil by ELISA methods [46,48].

Only a few studies applied immunoaffinity chromatography to the cleanup of samples for PCB detection. These studies used the classical Sepharose Ab-linked columns for purification of ^{13}C or ^{14}C dioxins and furans from serum [49–51] and 1,3,7,8-tetrachlorodibenzo-*p*-dioxin (1,3,7,8-TCDD) from bovine milk samples [52]. In all studies high binding efficiencies were achieved only after implementation of a solid-phase combined with a liquid–liquid extraction pre-affinity column cleanup step. Detection was performed by either GC–MS or liquid scintillation counting. Our ability to analyze the sol–gel immunoaffinity-purified samples by means of ELISA without addition of a pre- or post-cleanup step represents a novel major advantage in monitoring PCBs in environmental, food and biological samples.

5. Summary and conclusions

We have succeeded in developing several ELISAs in various formats for a few (representative coplanar) PCBs, i.e., PCB-77, PCB-126 and PCB-169, using goat anti-PCB-antisera or purified IgGs and a commercial anti-PCB-rabbit polyclonal Ab, with sensitivities that ranged from 0.1 to 63 ng mL^{-1}. The various Abs differed in their affinity toward the different PCBs: the goat anti-PCB IgGs exhibited the highest affinity and lowest detection limit for PCB-77, and the rabbit antiserum for PCB-169. The antisera or purified IgGs did not cross react with any other tested PCB, Aroclor of furan in any of the ELISA formats, with the exception of the goat IgGs, which recognized the heptachlorodibenzofuran 1,2,3,4,6,7,8-HpCDF.

In addition, we developed a sol–gel-based IAP method for sample cleanup of PCB-126. Measurement of recovery efficiencies revealed that the binding capacity differed among sol–gel formats, and that a format based on a TMOS:HCl ratio of 1:12 gave the highest binding capacity; other ratios, i.e., 1:4, 1:6 and 1:8 were much less efficient, exhibiting values lower than those obtained with the 1:12 format by factors of 4–8. Under the tested conditions the net binding capacity of the sol–gel IAP column ranged from 112 to 257 ng, and nearly 90% of the applied analyte could be eluted with only 2 mL of ethanol. The method has also proven to be very efficient in the cleanup of spiked soil/sediment sample extracts for PCB-126 detection; it eliminated matrix interferences to a degree that enabled analysis of the purified samples (equivalent to 2.5 mg of soil/sediment) by ELISA without the need of any additional pre- or post-IAP cleanup or extraction steps. Despite our success in applying the sol–gel IAP method to PCB-126, the method still needs optimization, especially with regard to the reduction of non-specific binding, and its efficiency in dealing with other PCBs and with soil/sediment samples from other sources, prepared by different extraction methods, needs to be evaluated.

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