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# Determination of polychlorinated biphenyls in soil and sediment by selective pressurized liquid extraction with immunochemical detection

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## HIGHLIGHTS

- A selective pressurized liquid extraction (SPLE) method was developed for analyzing PCBs.
- Aroclor and Coplanar PCB ELISAs were applied to the SPLE extracts.
- Soil and sediment samples from five different sites were analyzed using the SPLE–ELISA.
- SPLE–ELISA compared favorably with a conventional PCB multi-step analysis.
- SPLE–ELISA is useful for quantitative or qualitative analysis of PCBs.

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## ABSTRACT

A selective pressurized liquid extraction (SPLE) method was developed for a streamlined sample preparation/cleanup to determine Aroclors and coplanar polychlorinated biphenyls (PCBs) in soil and sediment. The SPLE was coupled with an enzyme-linked immunosorbent assay (ELISA) for an effective analytical approach for environmental monitoring. Sediment or soil samples were extracted with alumina, 10% $AgNO_3$ in silica, and sulfuric acid impregnated silica with dichloromethane at 100 °C and 2000 psi. The SPLE offered simultaneous extraction and cleanup of the PCBs and Aroclors, eliminating the need for a post-extraction cleanup prior to ELISA. Two different ELISA methods: (1) an Aroclor ELISA and (2) a coplanar PCB ELISA were evaluated. The Aroclor ELISA utilized a polyclonal antibody (Ab) with Aroclor 1254 as the calibrant and the coplanar PCB ELISA kit used a rabbit coplanar PCB Ab with PCB-126 as the calibrant. Recoveries of Aroclor 1254 in two reference soil samples were 92 ± 2% and 106 ± 5% by off-line coupling of SPLE with ELISA. The average recovery of Aroclor 1254 in spiked soil and sediment samples was 92 ± 17%. Quantitative recoveries of coplanar PCBs (107–117%) in spiked samples were obtained with the combined SPLE–ELISA. The estimated method detection limit was 10 ng $g^{-1}$for Aroclor 1254 and 125 pg $g^{-1}$ for PCB-126. Estimated sample throughput for the SPLE–ELISA was about twice that of the stepwise extraction/cleanup needed for gas chromatography (GC) or GC/mass spectrometry (MS) detection. ELISA-derived uncorrected and corrected Aroclor 1254 levels correlated well ($r = 0.9973$ and 0.9996) with the total Aroclor concentrations as measured by GC for samples from five different contaminated sites. ELISA-derived PCB-126 concentrations were higher than the sums of the 12 coplanar PCBs generated by GC/MS with a positive correlation ($r = 0.9441$). Results indicate that the SPLE–ELISA approach can be used for quantitative or qualitative analysis of PCBs in soil and sediments. To our knowledge, this is the first report of an SPLE–ELISA approach not requiring a post-extraction cleanup step for detecting Aroclors and coplanar PCBs in soil and sediment.
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## 1. Introduction

Polychlorinated biphenyls (PCBs) are synthetic organic compounds with 209 distinct congeners. PCBs are commonly used in capacitors and other electrical equipment because of their stability, insulating properties, and low burning capacity. PCBs were originally produced as specific mixtures of congeners known as Aroclors. The International Agency for Research on Cancer (IARC) classified PCBs as probable human carcinogens (2A group) (IARC., International Agency for

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Research on Cancer, 1987). Concern over the harmful ecological and human effects and the persistence of PCBs in the environment led the United States Congress to ban their domestic production in 1977. PCBs are still detected in various micro-environments (e.g., air, soil, dust, sediment, food, tissue) either as Aroclors or as individual congeners (ATSDR, Agency for Toxic Substances, Diseases Control Registry, 2000; Deng et al., 2002; Wilson et al., 2003; Kim et al., 2004; Sapozhnikova et al., 2004; Martinez et al., 2010). Human exposure to PCBs is through inhalation of contaminated air (outdoor or indoor), ingestion of contaminated food or non-food items, and dermal contact of contaminated surfaces. The primary route of exposure to PCBs is through consumption of contaminated lipid-enriched foods (e.g., fish and cooking oils) as PCBs can accumulate in these and other foodstuffs (ATSDR, Agency for Toxic Substances, Diseases Control Registry, 2000). PCB exposure has been associated with a variety of adverse health effects in humans, including hepatotoxicity, reproductive toxicity, reduced birth rate and neurodevelopmental disruption (ATSDR, Agency for Toxic Substances, Diseases Control Registry, 2000; Aoki, 2001; Schantz et al., 2003). They can affect the immune, reproductive, nervous, and endocrine systems, and have been linked to low intelligence quotients in children.

The analysis of PCBs in environmental samples is generally a multi-step process. Conventional methods including gas chromatography (GC) with electron capture detection (ECD) and/or mass spectrometry (MS) typically require a thorough sample cleanup (Muir and Sverko, 2006; US EPA, 2007, 2010). These methods are generally reliable and sensitive, however, they are time consuming, require tedious laboratory preparation steps and expensive equipment with highly trained personnel. The high costs for monitoring PCBs and related compounds are often a concern for regulatory agencies. Effective and low cost screening methods are needed for large-scale environmental monitoring and human exposure assessment programs. Sample extraction and cleanup are rate limiting factors for the overall throughput in PCB analysis of environmental and biological samples. Pressurized liquid extraction (PLE) is an automated, fast and efficient sample extraction technique that utilizes elevated temperatures and high pressures to achieve effective extraction of organic pollutants from solid matrices (Richter et al., 1996). PLE uses less solvent, and requires less time compared to the Soxhlet extraction employed in several methods for extracting solid samples (US EPA, 1994, 1996a). PLE techniques have been reported for the effective extraction of persistent organic pollutants including PCBs, dioxins, and furans from complex sample media (e.g., sediment, soil, tissue, oil), but required post-extraction cleanup of the extracts (Misita et al., 2003; Wilson et al., 2003; Robinson et al., 2004). Multi-step cleanup procedures such as acid wash, open-bed column chromatography, or gel permeation chromatography are required prior to GC or GC/MS. A streamlined sample preparation/cleanup strategy, of selective pressurized liquid extraction (SPLE) utilizing various adsorbents as an in-situ cleanup tool, was recently reported to retain fat and other co-extracted interferences during extraction of lipophilic contaminants including PCBs, polybrominated diphenylethers, dioxins, and furans from oil, feed, food, soil sediment, and tissue (Nording et al., 2005, 2006; Bjorklund et al., 2006; Haglund et al., 2007; Chuang et al., 2009; Zhang et al., 2011). SPLE incorporates cleanup adsorbents with the sample in an extraction cell for simultaneous extraction and cleanup of target analytes in complex matrices minimizing or completely eliminating the tedious cleanup steps prior to detection by either instrumental or immunochemical methods.

Immunochemical methods such as the enzyme linked immunosorbent assay (ELISA) typically provide advantages (e.g., lower cost, higher sample throughput) over GC methods for certain monitoring applications (Van Emon and Lopez-Avila, 1992, Van Emon, 2001, Van Emon et al., 2008a, 2008b). Immunochemical methods can easily be introduced into a chemical analysis laboratory and integrated with instrumental methods particularly for a tiered analytical approach (Van Emon et al., 2007). The U.S. EPA Office of Solid Waste has approved enzyme immunoassay methods for screening PCBs in soils and non-aqueous waste liquids (US EPA, 1996b) and for dioxins/furans in soils (US EPA, 2002). The use of various ELISA methods for the determination of PCBs in water, soil, and sediment has been reported (Franek et al., 1997, 2001; Johnson and Van Emon, 1996; Johnson et al., 2001; Lawruk et al., 1996; Chuang et al., 1998; Altstein et al., 2010; Bronshtein et al., 2012). In a previous study, sample matrix interferences were observed in a PCB ELISA that did not employ a post-extraction cleanup step. A more selective extraction procedure, supercritical fluid extraction (SFE) had to be developed to minimize the matrix interference (Johnson et al., 2001). However, SFE may not be suitable for the routine preparation of soil and sediment samples as it is not an exhaustive extraction procedure and is dependent on the physiochemical properties of the sample for efficient extraction. Samples from heterogeneous environmental sites may differ significantly and require extensive SFE method optimization per sample set. Post-extraction cleanup procedures are often required to minimize matrix interference by ELISA for the determination of lipophilic compounds such as PCBs, dioxins, furans, and polybrominated diphenylethers when more exhaustive extraction methods (e.g., Soxhlet extraction, PLE) are employed (Nichkova et al., 2004; Muir and Sverko, 2006 Shelver et al., 2008; Van Emon et al., 2008b). The addition of a cleanup step often reduces the advantages of low cost and high throughput of ELISA detection. These advantages can be maintained with the coupling of an effective single-step sample extraction/cleanup procedure such as SPLE with ELISA methods.

Described here is the development and evaluation of SPLE–ELISA methods for Aroclors and coplanar PCBS using contaminated soil and sediment samples with comparison to GC or GC/MS procedures. Contaminated sediment and soil samples from a field study conducted under an EPA Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) program (US EPA, 2004; Dindal et al., 2007) were analyzed using the optimal SPLE followed by an ELISA with specificity for either Aroclors or coplanar PCBs. The SPLE–ELISA results were compared with those obtained by conventional methods (stepwise extraction, cleanup and GC or GC/MS). The performance of the SPLE–ELISA technique was evaluated in terms of false positive and false negative rates, recovery, detection limit, method precision, sample throughput and appropriateness for environmental monitoring.

## 2. Experimental section

### 2.1. Samples

Two Aroclor standard reference soils (Environmental Resource Associates, Arvada, CO) and soil and sediment samples from a field study conducted under an EPA SITE MMT program (Dindal et al., 2007; US EPA, 2004) were used in the recovery experiments. Sediment and soil samples ($N = 32$) collected from five SITE MMT sampling sites were prepared by the SPLE–ELISA method for Aroclor 1254 and a subset of samples ($N = 10$) was used for coplanar PCB analysis.

### 2.2. Chemicals

Primary rabbit polyclonal (AC 3) anti-PCB antibodies (Abs) and a PCB coating antigen (Co-Ag 560-52 made by conjugating a PCB hapten to conalbumin) were previously prepared and described (Johnson and Van Emon, 1996). Goat anti-rabbit conjugated to horseradish peroxidase (HRP), mixed Aroclor standard solutions, alumina, phosphate buffered saline (PBS), PBS containing 0.1% (v/v) Tween-20 (PBST), and silver nitrate ($AgNO_3$) were obtained from Sigma (St. Louis, MO). Coplanar PCB standards were obtained from Cambridge Isotope Laboratories (Andover, MA). One-step, Ultra 3,3′,5,5′-tetramethylbenzidine (TMB) ELISA substrate was purchased from Pierce (Rockford, IL). Coplanar PCB ELISA testing kits were purchased from Abraxis (Warminster, PA). Dichloromethane (DCM), ethyl ether (EE), hexane, methanol, toluene,

distilled-in-glass grade, and Florisil solid phase extraction (SPE) columns were purchased from VWR (West Chaster, PA). Glass fiber PLE filters were from Dionex (Sunnyvale, CA). Silica (100–200 mesh, grade 60A or equivalent) was purchased from Fisher Scientific (Fair Lawn, NJ). Hydromatrix was purchased from Varian (Walnut Creek, CA).

### 2.3. SPLE

All extractions were performed using a Dionex Accelerated Solvent Extraction 200 system (Sunnyvale, CA). Different combinations of adsorbents were evaluated based on the SPLE procedure previously developed for dioxins and furans (Chuang et al., 2009). The final SPLE method for PCBs was to mix an aliquot (4 g) of each sample with Hydromatrix (3 g), prior to placement in a 33 mL extraction cell. The bottom of the extraction cell was covered with a glassfiber filter, followed by 3 g of alumina, 1 g of 10% $AgNO_3$ in silica, and 6 g of sulfuric acid impregnated silica (acid silica) as shown in Fig. 1 (Chuang et al., 2009; US EPA, 2010). The sample mixture was next placed in the extraction cell followed by clean sand to completely fill the cell. The extraction was carried out at 100 °C, with a purge time of 60 s, a flush volume of 100%, and an extraction time of 10 min and 3 cycles. The resulting extracts were concentrated for subsequent analysis. An aliquot of the sample extract was solvent exchanged from DCM to methanol and diluted with PBST (40% methanol in PBST) for the Aroclor ELISA. An aliquot of the DCM extract was solvent exchanged into methanol and diluted with reagent water (50% methanol in water) for the coplanar PCB ELISA. Additional dilutions were performed on the samples as necessary using the respective assay buffers.

### 2.4. Stepwise PLE and cleanup

Aliquots of sediment and soil samples were extracted with DCM using PLE (Misita et al., 2003) without any cleanup adsorbents. A multi-step cleanup procedure was used for the DCM extracts prior to GC/ECD analysis for Aroclors and GC/MS analysis for coplanar PCBs. The DCM extracts were concentrated and fractionated by gel permeation chromatography (GPC) to isolate the PCBs from other contaminants. The target fraction was solvent exchanged into hexane and applied to a preconditioned Florisil SPE column, with 50% EE in hexane and 100% hexane. The fraction eluted with 15% EE in hexane and was concentrated for subsequent analysis (Wilson et al., 2003).

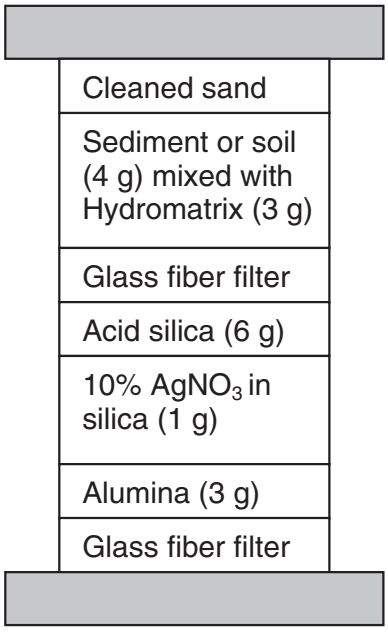


**Fig. 1.** Packing of the extraction cell.

### 2.5. ELISA analysis

#### 2.5.1. Aroclor ELISA

Microplates (Nunc MaxiSorp ELISA plates) were coated with 100 μL of the Co-Ag 560-52 conjugate, diluted 1:40,000 (containing 10 ng per 100 μL) in 0.5 M carbonate buffer, pH 9.6 and incubated overnight at 4 °C. After the incubation, microwells were washed three times with PBST. Next, 50 μL aliquots each of: Aroclor 1254 (ranging from 0.096 to 200 ng $mL^{-1}$ diluted in PBST/40% methanol), sediment or soil sample extracts in 40% methanol in PBST (5 serial dilutions), and QC samples (5 serial dilutions ranging from 6.44 to 100 ng $mL^{-1}$) were added to the wells followed by the addition of 50 μL of polyclonal (AC-3) anti-PCB primary antibodies diluted 1:3000 in PBST (final dilution 1:6000). In addition, four microwells received only 40% methanol in PBST and served to determine maximal binding in the absence of the competing antigen, which was designated as 100%. Four other microwells received a ten-fold excess of the Aroclor 1254 (2000 ng $mL^{-1}$) in 40% methanol in PBST and served as a control to determine non-specific binding. Plates were incubated for 3 h at room temperature; washed three times with PBST; and 100 μL of a secondary antibody (goat anti rabbit conjugated to HRP, diluted 1:30,000 in PBST) was added. Plates were incubated for 2 h at room temperature. At the end of the incubation, plates were washed with PBST and 100 μL of 1-Step Ultra TMB-ELISA substrate was added to the wells. The reaction was stopped after 10–20 min by the addition of 50 μL of 4 M sulfuric acid. The absorbance at 450 nm was measured with a Lucy 2 microplate reader (Anthos, Eugendorf, Austria). The content of Aroclor 1254 was determined from an Aroclor 1254 calibration curve after linearization of the data by transformation to a logit-log plot by means of Microcal Origin software (Bronshtein et al., 2012).

#### 2.5.2. Coplanar PCB ELISA

This ELISA was performed using a coplanar-PCB testing kit which contained all the necessary immunoreagents. The coplanar PCB calibration standard solutions, quality control (QC) samples, and sediment and soil samples were analyzed in duplicate for each assay run. An aliquot (50 μL) of rabbit anti-coplanar PCB antibody was added to each microtiter well coated with goat-anti rabbit antibody. An aliquot (50 μL) of each calibration solution (0, 25, 50, 100, 250, 500, 1000 pg $mL^{-1}$ of PCB-126), negative and positive control solutions, and sample extracts were added to each appropriate well and incubated at room temperature for 30 min. After incubating, an aliquot (50 μL) of the coplanar PCB–HRP enzyme conjugate solution was added to each microwell, the plate was covered and incubated at room temperature for 90 min. After the incubation, the content of the wells were discarded into a waste container. The plate was washed three times with 3 × 250 μL of the washing buffer solution. Any remaining wash buffer solution in the wells was removed by patting the plate on a dry stack of paper towels. After the final wash, an aliquot (150 μL) of the chromogenic substrate solution was added to the plate. The plate was covered and allowed to incubate at room temperature for 25 min. At the end of the incubation, an aliquot (50 μL) of an acidic stopping solution was added, and each microwell was read using a Molecular Devices Spectra Max Plus microplate spectrophotometer (Sunnyvale, CA). The absorbance of the microwells was determined at 450 nm. Data processing was performed with SOFTMaxPro software version 4.6 interfaced to a personal computer using a 4-parameter curve fit.

### 2.6. GC analysis

The samples and standard solutions were analyzed by GC with ECD for Aroclor concentrations based on EPA Method 8082A (US EPA, 2007). The GC column was a DB-5 fused silica capillary column

(60 m × 0.25 mm, 0.25 μm film thickness), and hydrogen was used as the carrier gas. The initial GC temperature was 60 °C for 1 min and programmed to 140 °C at 10 °C/min; from 140 °C to 220 °C at 0.9 °C/min; from 220 °C to 290 °C at 5 °C/min; and held at 290 °C for 10 min. Identification and quantification were accomplished by integrating representative major peaks in the Aroclor standard, and identifying and integrating those same peaks (by retention time and pattern matching) in the samples (US EPA, 2007).

### 2.7. GC/MS analysis

The target fractions and standards (coplanar PCBs) were analyzed by 70 eV electron impact GC/MS. A Hewlett-Packard GC/MS was operated in the selected ion monitoring (SIM) mode. Data acquisition and processing were performed with a ChemStation data system. The GC/MS procedure was based on key components of the PCB congener analysis approach described in EPA Method 1668C (US EPA, 2010). Overall guidance for the method is based on EPA Method 8270D (US EPA, 2006). The GC column was a DB-XLB fused silica capillary (60 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the GC carrier gas. Following injection, the GC column was set at 60 °C for 1 min, temperature programmed to 140 °C at 10 °C/min, at 0.9 °C/min to 220 °C/min, and at 5 °C/min to 290 °C (hold for 15 min). Peaks monitored were the molecular ion peaks and their associated characteristic fragment ion peaks. Identification of the target PCBs was based on their GC retention times relative to the internal standard (IS) and the relative abundances of the monitored ions. Quantification was performed by comparing the integrated ion current response of the target ions to those of the IS. The average response factors of the target ions were generated from the standard calibrations.

### 2.8. Data analysis

Spike recovery data were calculated based on the difference between the Aroclor 1254 or coplanar PCB measurements in the corresponding spiked and non-spiked samples. For reference soil samples, recovery data were calculated based on the expected values of the soil samples. The Aroclor ELISA was calibrated against Aroclor 1254. The ELISA result integrates the effects of other Aroclors and multiple PCB-like compounds with various cross reactivity (CR) and gives a single Aroclor 1254 equivalent (EQ) value. Similarly, the coplanar PCB ELISA-derived result includes compounds similar to PCB-126 that is reported as a PCB 126 EQ value. The SPLE ELISA-derived Aroclor 1254 EQ and the sums of the stepwise PLE GC-derived Aroclor concentrations (the sums of Aroclors 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262) were used for method validation. Similarly, for the coplanar PCB ELISA, the ELISA derived PCB-126 levels were compared with the sums of 12 coplanar PCBs by GC/MS. The non-detectable values were replaced with one-half the detection limit to calculate descriptive statistics for characterizing the distribution of results for each method. Sample size, arithmetic mean, standard deviation, geometric mean, range and percentiles were calculated. The Pearson correlation coefficient measuring the extent of linear agreement between the ELISA and GC/MS data was also calculated. The GC derived Aroclor concentrations were considered as a reference value in calculating false negative and false positive rates for the SPLE–ELISA method at four concentration levels (i.e., 100, 1000, 10,000, and 100,000 ng $g^{-1}$).

## 3. Results and discussion

### 3.1. Evaluation of SPLE for PCBs

An effective SPLE procedure should quantitatively extract the target analyte(s) from the sample matrix, and reduce the co-extracted materials, to eliminate the post-extraction cleanup prior to detection. Previously several SPLE procedures were evaluated using various combinations of adsorbents for an in situ cleanup and extraction of dioxins and furans in environmental samples (Chuang et al., 2009). The optimal SPLE conditions for dioxins and furans were evaluated for the quantitative removal of PCBs in the contaminated soil and sediment matrices. This SPLE procedure was evaluated based on GC/ECD data for Aroclor 1254 and GC/MS data for the coplanar PCBs. Recovery data showed that the SPLE procedure consisting of extracting soil or sediment together with alumina, 10% $AgNO_3$ in silica, and acid silica using DCM as the solvent at 100 °C and 2000 psi provided the cleanest extracts and the best recoveries for both Aroclor 1254 and coplanar PCBs. Quantitative recoveries of Aroclor 1254 were achieved for the two reference soil samples (95–101%) as well as the spiked sediment samples (88–104%) by GC/ECD. Satisfactory recoveries of PCB-77, PCB-126, and PCB-169 were also achieved in the spiked soils (85–104%) and sediments (90–120%) using the optimal SPLE with GC/MS. Only one sample required a post-extraction cleanup. These findings suggested that the SPLE procedure effectively removed PCBs from the soil and sediment samples without extracting any interfering substances. Thus, this particular SPLE procedure was selected for additional evaluation experiments for off-line coupling with ELISA detection.

### 3.2. ELISA method performance

#### 3.2.1. Aroclor ELISA

The quantitative Aroclor ELISA previously developed by the EPA NERL was optimized for the SITE MMT samples (Johnson and Van Emon, 1996). Checkerboard titration experiments were performed to determine the optimal concentrations of the polyclonal anti-PCB Ab, coating antigen, and the secondary antibody-enzyme conjugate. The optimal conditions established for the Aroclor ELISA were: a dilution of 1:40,000 of the coating antigen (Co-Ag 560-52 conjugate), a dilution of 1:6000 of anti PCB antibody and a dilution of 1:30,000 of the antibody–enzyme conjugate (goat anti rabbit HRP). Triplicate analyses were conducted for each standard or sample extract by ELISA and the means of the triplicate values were used to calculate the final concentrations. The analyte diluent previously established in the Aroclor ELISA was 30% methanol in PBST (15% methanol in PBST as the final assay concentration) (Johnson and Van Emon, 1996). Additional investigations were performed in this study to determine if the assay could tolerate more methanol to accommodate the SITE MMT samples. Results showed that the presence of methanol in PBST (up to 50% final assay concentration for Aroclor 1254 and 25% for Aroclor 1248) did not significantly affect the $I_{50}$ and $I_{20}$ values. A final concentration of 20% methanol was used as a safety factor for assay performance. The sample extracts and standard solutions were thus prepared in 40% methanol in PBST with Aroclor 1254 as a calibrant. The average $I_{50}$ value for Aroclor 1254 was 7.5 ± 1.0 ng $mL^{-1}$ (N = 8) which is similar to that obtained previously (10 ng $mL^{-1}$) with 15% methanol. Day-to-day consistency was observed for the shape of the calibration curves. Percent standard deviation for the 100 ng $mL^{-1}$ QC samples analyzed in different days was within 17% (107 ± 18 ng $mL^{-1}$). The estimated assay detection limit for Aroclor 1254 based on the $I_{20}$ value was 1.8 ± 0.8 ng $mL^{-1}$ (N = 8). Examination of the cross reactivity (CR) in the Aroclor 1254 assay revealed CR values for other Aroclors as 76% for 1016 and 1242, 47% for 1248, 41% for 1262, 35% for 1260 and 13% for 1232. No CR was detected with either Aroclors 1221, 1268, or coplanar PCBs (PCB-77, PCB-126, PCB-169).

#### 3.2.2. Coplanar PCB ELISA

The coplanar PCB ELISA was performed following the instructions provided by the testing kit. The kit is based on using duplicate analyses with the means of the duplicate values used to calculate the final concentrations. The % relative difference (%D) values of the duplicate analyses ranged from 7.5 to 30% for standard solutions and sample extracts. Day-to-day variation of the ELISA expressed as percent relative standard deviation (%RSD) of the $I_{50}$ values was within ±15% (524 ± 73 pg $mL^{-1}$). The $R^2$ value of each calibration curve was

greater than 0.99. Recoveries of the back-calculated standard solutions were greater than 80% of the expected values. Negative control solutions (0 pg $mL^{-1}$) were below the assay detection limit (25 pg $mL^{-1}$). Quantitative recoveries (82–129%) were also obtained for the positive control solutions (50–500 pg $mL^{-1}$). CR values provided by the ELISA kit were 100% for PCB-126, 300% for PCB-169, 5.3% for PCB-77, 3% for PCB-189, 2.7% for PCB-81, and less than 1% for the remaining seven coplanar PCBs (0.5–0.07%). The coplanar PCB ELISA had very low CRs toward Aroclors (<0.1%).

### 3.3. SPLE–ELISA performance

SPLE–ELISA spike recovery experiments were performed using different aliquots of soil and sediment samples extracted with the optimal SPLE conditions for Aroclor ELISA and coplanar PCB ELISA. Post-extraction cleanup was not required in any of the samples prior to the Aroclor ELISA or coplanar PCB ELISA. Recoveries for Aroclor 1254 were 95 ± 2% and 106 ± 5% of the expected concentrations in the two reference soils. Some of the soil and sediment samples contained dioxins and furans as indicated by GC/MS (Chuang et al., 2009). The analysis of these samples by the SPLE–ELISA approach indicated that dioxins and furans did not interfere based on Aroclor 1254 recoveries of the spiked soil and sediment samples ranging from 64 to 112% with an average of 92 ± 17%. The percent difference (%D) concentrations in duplicate aliquots of soil and sediment samples ranged from 0 to 7.6% with the exception of one sample (%D = 47%). The greater variation observed with the real-world sample could be due to sample heterogeneity. Samples were mixed by manual stirring prior to removing each aliquot. No heterogeneity determinations were made. Sample extracts were analyzed by ELISA at different dilutions, and similar results (%RSD within ±30%) were obtained indicating negligible sample matrix interference. Analysis of method blanks (using cleaned sand as a sample and respective adsorbents) did not detect any Aroclor 1254. The estimated method detection limit for Aroclor 1254 using the SPLE–ELISA was 10 ng $g^{-1}$ (4 g sample), with 10% of the DCM sample extract solvent exchanged into 1 mL of 40% methanol in PBST for ELISA. Satisfactory recoveries of PCB-126 were obtained in the spiked soil (117 ± 2%) and sediment (107 ± 22%) samples. The %D of duplicate samples ranged from 4 to 19%. The estimated method detection limit for PCB-126 in the SPLE–ELISA was 125 pg $g^{-1}$. Method blanks were also analyzed by the SPLE–ELISA and yielded non-detectable values.

### 3.4. Comparison of SPLE–ELISA and the stepwise PLE/cleanup-GC procedure

For method validation, thirty two soil and sediment samples were prepared by the SPLE and analyzed by the Aroclor ELISA. Note that the differences between the ELISA CRs on various Aroclors could lead to differences between the ELISA and the GC derived Aroclor data. A sample highly contaminated with Aroclor 1260 from a PCB landfill site gave the maximum response for both GC (727,250 ng $g^{-1}$) and ELISA (corrected data 401,786 ng $g^{-1}$) methods. In addition, the difference between the two methods could be due to the heterogeneity of the sample aliquots or different sample preparation steps. Thus, for samples containing Aroclors other than Aroclor 1254 (GC results), the corrected ELISA data were generated by the respective CRs of other detected Aroclors for comparison. Summary statistics for the ELISA and GC results are shown in Table 1. Both non-corrected and corrected ELISA data are reported. In addition to similar geometric means, similar Aroclor concentrations were observed in the 25th, 50th, 75th, and 90th percentiles between the two methods. Generally, there was a strong and positive relationship between the ELISA (both non-corrected and corrected) and GC data. The correlation between the two methods was not significantly influenced by this heavily contaminated sample as evidenced by a Pearson correlation coefficient of r = 0.9973 (non-corrected ELISA data vs. GC data) and 0.9996 (corrected ELISA data vs. GC data) for 32 samples versus r = 0.9184 and 0.9778 by removing this data pair. Fig. 2 displays the relationship between the corrected ELISA and GC data.

Table 2 summarizes the number and percentage of the soil and sediment samples that fall within each of the four categories denoted by whether or not the reported sample concentrations were at or above a specified threshold for either method. If the GC procedure represents a standard method, the false positive rate for the samples was 0% for the SPLE–ELISA method at the comparative levels of 1000, 10,000, and 100,000 ng $g^{-1}$ and increased to 16% at the level of l00 ng $g^{-1}$ level. The false negative rate was 0% at the levels of 1000 and 100,000 ng $g^{-1}$ and 3% at the levels of 100 and 10,000 ng $g^{-1}$. Note that the false negative rate at 10,000 ng $g^{-1}$ was reduced to 0% if the corrected ELISA data were used.

Different aliquots of a sample subset (N = 10) were extracted by the SPLE procedure and analyzed by the coplanar PCB ELISA. Summary statistics for ELISA and GC/MS data are shown in Table 3. The ELISA-derived PCB-126 EQ concentrations were higher than the sums of the 12 coplanar PCBs measured by GC/MS. The higher ELISA-derived PCB-126 EQ data could be due to the CR to other PCB congeners and/or PCB-like compounds that are not measured by the GC/MS method. The ELISA and GC/MS data are highly correlated with a correlation coefficient of 0.9441.

The SPLE–ELISA method and the conventional stepwise extraction/cleanup method using either GC/ECD or GC/MS detection had similar overall method precision and detection limits for the soil and sediment samples containing Aroclors or coplanar PCBs. The SPLE–ELISA had a higher sample throughput as a cleanup step was not required which also reduced the overall analysis costs.

## 4. Conclusions

An SPLE method was developed that provided a streamlined sample preparation/cleanup procedure for the immunochemical detection of PCBs in environmental samples. An Aroclor ELISA and a coplanar PCB ELISA were both evaluated for use with the SPLE method. Aroclor 1254 and PCB-126 were used as calibration standards for the 96-micro well

**Table 1**
Summary statistics for ELISA Aroclor 1254 EQ and GC/ECD Aroclor data.

| Summary statistics[a] | Uncorrected ELISA Aroclor 1254 EQ, | Corrected ELISA Aroclor 1254 EQ, ng $g^{-1}$ | GC Aroclors, ng $g^{-1}$ ng $g^{-1}$ |
|---|---|---|---|
| Arithmetic mean | 5674 | 14,343 | 24,260 |
| Standard deviation | 24,742 | 70,798 | 128,324 |
| Geometric mean | 233 | 265 | 202 |
| Minimum | nd[b] | nd[b] | nd |
| 25th percentile | 66.4 | 66.4 | 32.3 |
| 50th percentile | 141 | 141 | 113 |
| 75th percentile | 1503 | 1503 | 1571 |
| 90th percentile | 6694 | 7166 | 6463 |
| Maximum | 140,625 | 401,786 | 727,250 |

[a] Sample size = 32
[b] nd denotes not detected; estimated detection limit was 10 ng $g^{-1}$.

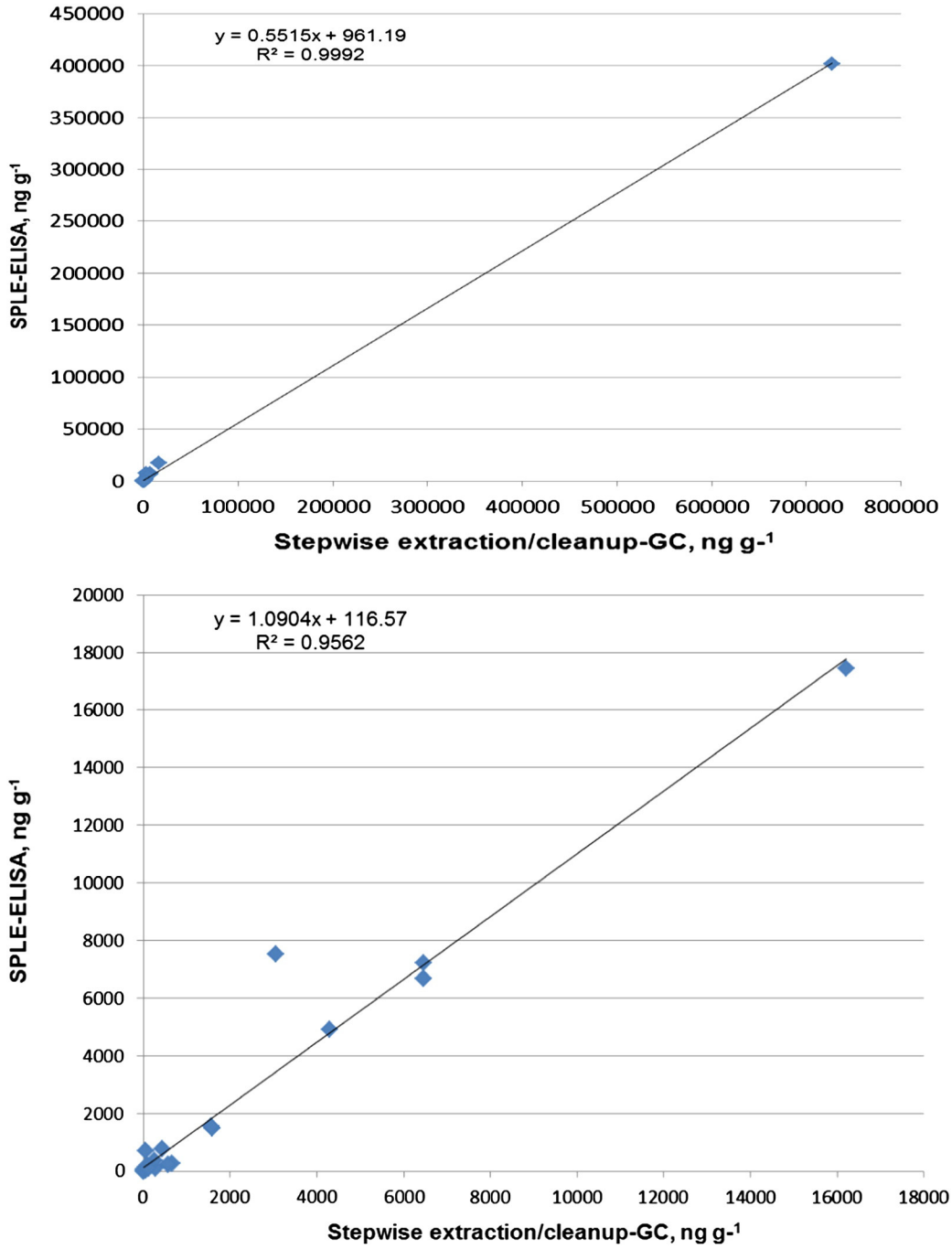


**Fig. 2.** Data comparison of corrected Aroclor 1254 EQ by SPLE–ELISA and the sums of Aroclors by stepwise extraction/cleanup-GC. The upper graph includes all data (n = 32). In the lower graph the most contaminated sample is eliminated (n = 31), allowing for an expanded view of all other samples.

**Table 2**
ELISA and GC/MS classification of soil and sediment samples at or above comparative concentrations.

| Comparative concentration, ng $g^{-1}$ | Number (%) of 32 soil and sediment samples with[a]: | | | |
|---|---|---|---|---|
| | ELISA ≥ conc.; GC < conc. (false positive) | ELISA < conc.; GC ≥ Conc. (false negative) | Both ELISA and GC ≥ conc. (true positive) | Both ELISA and GC < conc. (true negative) |
| 100 | 6 (16%) | 1 (3%) | 14 (44%) | 12 (38%) |
| 1000 | 0 (0%) | 0 (0%) | 9 (28%) | 23 (72%) |
| 10,000 | 0 (0%) | 1 (3%) | 1 (3%) | 30 (94%) |
| 100,000 | 0 (0%) | 0 (0%) | 1 (3%) | 31 (97%) |

[a] Non-corrected ELISA data were used.

**Table 3**
Summary statistics for ELISA PCB-126 EQ and GC/MS coplanar PCB data.

| Summary statistics[a] | ELISA PCB-126 EQ, ng $g^{-1}$ | GC/MS Coplanar PCBs, ng $g^{-1}$ |
|---|---|---|
| Arithmetic Mean | 37.6 | 19.6 |
| Standard Deviation | 51.9 | 37.5 |
| Geometric Mean | 16.2 | 4.91 |
| Minimum | 3.30 | 1.02 |
| 25th Percentile | 4.68 | 1.27 |
| 50th Percentile | 15.3 | 4.01 |
| 75th Percentile | 53.4 | 7.73 |
| 90th Percentile | 94.9 | 66.0 |
| Maximum | 165 | 116 |

[a] Sample size = 10

ELISAs. Quantitative recoveries were achieved with two reference soils using Aroclor 1254 as a calibration standard with an estimated detection limit of 10 ng $g^{-1}$ for Aroclors. Quantitative recoveries were obtained for spiked soil and sediment samples using PCB-126 as the calibrant with an estimated detection limit of 125 pg $g^{-1}$. The SPLE–ELISA sample throughput was more than twice that of the conventional analytical methods (e.g., step-wise extraction/cleanup and GC or GC/MS detection) and the overall costs were lower. The ELISA Aroclor 1254 EQ and the GC Aroclor results were linearly correlated for the 32 sediment and soil samples. Similarly the ELISA PCB-126 EQ and the GC/MS coplanar PCB data were correlated for the 10 sediment and soil samples. The study results suggest that an SPLE–ELISA approach offers application as either a low-cost qualitative or quantitative method for monitoring Aroclor 1254. The Aroclor 1254 ELISA could be calibrated with a mixture of Aroclors matching the characterized Aroclor pattern from sites containing mixed Aroclors. The coplanar PCB ELISA can provide a qualitative measure for coplanar PCBs at contaminated waste sites. The combination of SPLE–ELISA can also be utilized in a tiered approach for the low-cost qualitative screening of samples in environmental field studies prior to more costly GC Aroclor-specific or GC/MS PCB congener-specific detection methods. For many environmental monitoring applications, SPLE in tandem with ELISA offers cost and time advantages particularly for large scale field studies. The environmental data reported here using SPLE–ELISA included a wide range of Aroclors (<10 ng $g^{-1}$ to over 400,000 ng $g^{-1}$) further indicating the utility of this approach for monitoring both background levels and highly contaminated sites.

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