To be submitted to Science of the Total Environment

Determination of Polychlorinated Biphenyls in Soil and Sediment by Selective Pressurized Liquid Extraction with Immunochemical Detection

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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 100ºC 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 the SPLE-ELISA approach can be used for quantitative or qualitative analysis of PCBs in soil and sediments.

Keywords: Selective pressurized liquid extraction; Enzyme-linked immunosorbent assay; PCB; Aroclor; Coplanar PCB; Sediment; Soil

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, 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, 2000; Deng et al., 2002; Wilson et al., 2003; Kim et al., 2004; Sapozhnikova et al., 2004; Martinez et al., 2010). Human exposures 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, 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, 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 et al., 2006; US EPA, 2007 and 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 programs. Sample extraction and cleanup are rate limiting factors for sample 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 and 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 and 2006; Bjorklund et al., 2006; Haglund et al., 2007; Chuang et al., 2009; Zhang et al., 2011). SPLE incorporates cleanup absorbents 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, Lopez-Avila 1992, Van Emon 2001, Van Emon et al., 2008a and 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). 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 and 2001; Johnson, Van Emon 1996 Johnson et al., 2001; Lawruk et al., 1996; Chuang et al., 1998; Altstein, et al., 2010; Bronshetin 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 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, 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 ELISA 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, and sample throughput.

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 polyclonal (AC 3) anti-PCB antibodies (Abs) and the conjugate, Co-Ag 560-52 were obtained from the EPA (Johnson, 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 absorbents 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 Figure 1 (Chuang et al., 2009; US EPA, 2010). The sample mixture was next placed in the extraction cell followed by cleaned sand to completely fill the cell. The extraction was carried out at 100ºC, 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

159 reagent water (50% methanol in water) for the coplanar PCB ELISA. Additional dilutions were
160 performed on the samples as necessary using the respective assay buffers.

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162 *2.4 Stepwise PLE and cleanup*

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164 Aliquots of sediment and soil samples were extracted with DCM using PLE (Misita et al., 2003)
165 without any cleanup absorbents. A multi-step cleanup procedure was used for the DCM extracts
166 prior to GC/ECD analysis for Aroclors and GC/MS analysis for coplanar PCBs. The DCM extracts were
167 concentrated and fractionated by gel permeation chromatography (GPC) to isolate the PCBs
168 from other contaminants. The target fraction was solvent exchanged into hexane and applied to a
169 preconditioned Florisil SPE column, with 50% EE in hexane and 100% hexane. The fraction eluted with
170 15% EE in hexane and was concentrated for subsequent analysis (Wilson et al., 2003).

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172 *2.5 ELISA analysis*

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174 *2.5.1 Aroclor ELISA*

175 Microplates (Nunc MaxiSorp ELISA plates) were coated with 100 µL of the Co-Ag 560-52
176 conjugate, diluted 1:40,000 (containing 10 ng per 100 µL) in 0.5 M carbonate buffer, pH 9.6 and
177 incubated overnight night at 4°C. After the incubation, microwells were washed three times with PBST.
178 Next, 50 µL aliquots of Aroclor 1254 (ranging from 0.096 to 200 ng mL^{-1} diluted in PBST/40%
179 methanol), sediment or soil sample extracts in 40% methanol in PBST (5 serial dilutions), and QC
180 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 1:3,000 in PBST (final dilution 1:6,000). 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) were 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 were 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

The 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 the appropriate well and incubated at room temperature for 30 minutes. After incubating, an aliquot (50 µL) of the coplanar PCB labeled with 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 x 250 µL of the washing buffer

206 solution. Any remaining wash buffer solution in the wells was removed by patting the plate on a dry
207 stack of paper towels. After the final wash, an aliquot (150 µL) of the chromogenic substrate solution
208 was added to the plate. The plate was covered and allowed to incubate at room temperature for 25 min.
209 At the end of the incubation, an aliquot (50 µL) of an acidic stopping solution was added, and each
210 microwell was read using a Molecular Devices Spectra Max Plus microplate spectrophotometer
211 (Sunnyvale, CA). The absorbance of the microwells was determined at 450 nm. Data processing was
212 performed with SOFTMaxPro software version 4.6 interfaced to a personal computer using a 4-
213 parameter curve fit.

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215 *2.6 GC Analysis*

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217 The samples and standard solutions were analyzed by GC with ECD for Aroclor concentrations
218 based on EPA Method 8082A (US EPA, 2007). The GC column was a DB-5 fused silica capillary
219 column (60m x 0.25 mm, 0.25 µm film thickness), and hydrogen was used as the carrier gas. The initial
220 GC temperature was 60°C for 1 min and programmed to 140°C at 10°C /min; from 140°C to 220°C at
221 0.9°C/min; from 220°C to 290°C at 5°C/min; and held at 290°C for 10 min. Identification and
222 quantification were accomplished by integrating representative major peaks in the Aroclor standard, and
223 identifying and integrating those same peaks (by retention time and pattern matching) in the samples
224 (US EPA, 2007).

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226 *2.7 GC/MS Analysis*

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The target fractions and standards (coplanar PCBs) were analyzed by 70eV 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 (60m x 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^{o}C for 1 min, temperature programmed to 140^{o}C at 10^{o}C/min, at 0.9^{o}C/min to 220^{o}C/min, and at 5^{o}C/min to 290^{o}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 other PCB-126 like compounds and reported as 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. Descriptive statistics were calculated to characterize the distribution of results for each method. The non-detectable values were replaced with one-half the detection limit. 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, 10000, and 100000 ng g^{-1}).

3. Results and discussion

3.1 Evaluation of SPLE for PCBs

The SPLE procedure recently developed for dioxins and furans in contaminated soil and sediment matrices (Chuang et al., 2009) together with other combinations of absorbents and PLE extraction temperatures were tested for quantitative removal of PCBs in the contaminated soil and sediment matrices. The SPLE procedure was initially 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 100ºC 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

277 required a post-extraction cleanup. These findings suggested that the SPLE procedure effectively
278 removed PCBs from the soil and sediment samples without extracting any interfering substances. Thus,
279 this particular SPLE procedure was selected for additional evaluation experiments for off-line coupling
280 with ELISA detection.
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283 *3.2 ELISA methods performance*
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285 *3.2.1 Aroclor ELISA*
286 The optimization of the Aroclor ELISA was based on the quantitative Aroclor ELISA previously
287 developed by the EPA NERL (Johnson, Van Emon 1996). Checkerboard titration experiments were
288 performed to determine the optimal concentrations of the polyclonal (AC-3) anti-PCB Ab, coating
289 antigen, and the antibody-enzyme conjugate. The optimal conditions established for the Aroclor ELISA
290 were: a dilution of 1:40,000 of the coating antigen (Co-Ag 560-52 conjugate), a dilution of 1:6000 of
291 anti PCB antibody and a dilution of 1:30,000 of the antibody-enzyme conjugate (goat anti rabbit HRP).
292 Triplicate analyses were conducted for each standard or sample extract by ELISA and the means of the
293 triplicate values were used to calculate the final concentrations. The analyte diluent previously
294 established in the Aroclor ELISA was 30% methanol in PBST (15% methanol in PBST as the final
295 assay concentration) (Johnson, Van Emon 1996). Additional investigations were carried out in this
296 study to determine if the assay could tolerate more methanol to accommodate the lipophilic nature of
297 PCBs. Results showed that the presence of methanol in PBST (up to 50% as final assay concentration)
298 did not significantly affect the Aroclor 1254 assay I_{50} and I_{20} values and the methanol tolerance for
299 Aroclor 1248 assay was about 25%. Even though the assay tolerates up to 50% of methanol we chose to
300 work with 20% methanol. The sample extracts and standard solutions were prepared in 40% methanol in
301 PBST resulting in the final assay concentration as 20% methanol in PBST and using Aroclor 1254 as a
302 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 with 15% methanol in final assay concentration (10 ng mL^{-1}). 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} was 1.8 ± 0.8 ng mL^{-1} (N=8). Examination of cross reactivity (CR) with Aroclor 1254 as a reference 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 Aroclors 1221, 1268, and 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. Duplicate analyses were performed and the means of the duplicate values were 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 PCB77, 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 for Aroclor ELISA and coplanar PCB ELISA. Post-

329 extraction cleanup was not required in any of the samples prior to the Aroclor ELISA or coplanar PCB
330 ELISA. Recoveries for Aroclor 1254 were 95±2% and 106±5% of the expected concentrations in the
331 two reference soils. Aroclor 1254 recoveries of the spiked soil and sediment samples ranged from 64 to
332 112% with an average of 92±17%. The percent difference (%D) concentrations in duplicate aliquots of
333 soil and sediment samples ranged from 0 to 7.6% with the exception of one sample (%D = 47%). The
334 greater variation observed with the real-world sample could be due to sample heterogeneity. Samples
335 were mixed by manual stirring prior to removing each aliquot. No heterogeneity determinations were
336 made. Sample extracts were analyzed by ELISA at different dilutions, and similar results (%RSD within
337 ±30%) were obtained indicating negligible sample matrix interference. Analysis of method blanks
338 (using cleaned sand as a sample and respective adsorbents) did not detect any Aroclor 1254. The
339 estimated method detection limit for Aroclor 1254 using the SPLE-ELISA was 10 ng g^{-1} (4 g sample),
340 with 10% of the DCM sample extract solvent exchanged into 1 mL of 40% methanol in PBST for
341 ELISA. Satisfactory recoveries of PCB-126 were obtained in the spiked soil (117±2%) and sediment
342 (107±22%) samples. The %D of duplicate samples ranged from 4 to 19%. The estimated method
343 detection limit for PCB-126 using the SPLE-ELISA was 125 pg g^{-1}. Method blanks were also analyzed
344 by the SPLE-ELISA and yielded non-detectable values.
345
346 *3.4 Comparison of SPLE-ELISA and the stepwise PLE/cleanup-GC procedure*
347
348 For method validation, thirty two soil and sediment samples were prepared by the SPLE and
349 analyzed by the Aroclor ELISA. Note that the differences between the ELISA CRs on various Aroclors
350 could lead to differences between the ELISA and the GC derived Aroclor data. A sample highly
351 contaminated with Aroclor 1260 from a PCB landfill site gave the maximum response for both GC
352 (727250 ng g^{-1}) and ELISA (corrected data 401786 ng g^{-1}) methods. In addition, the difference between
353 the two methods could be due to the heterogeneity of the sample aliquots or different sample preparation
354 steps. Thus, for samples containing Aroclors other than Aroclor 1254 (GC results), the corrected ELISA

355 data were generated by the respective CRs of other detected Aroclors for comparison. Summary
356 statistics for the ELISA and GC results are shown in Table 1. Both non-corrected and corrected ELISA
357 data are reported. In addition to similar geometric means, similar Aroclor concentrations were observed
358 in the 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles between the two methods. Generally, there was a strong and
359 positive relationship between the ELISA (both non-corrected and corrected) and GC data. The
360 correlation between the two methods was not significantly influenced by this heavily contaminated
361 sample as evidenced by a Pearson correlation coefficient of r = 0.9973 (non-corrected ELISA data vs.
362 GC data) and 0.9996 (corrected ELISA data vs. GC data) for 32 samples versus r = 0.9184 and 0.9778
363 by removing this data pair. Figure 2 displays the relationship between the corrected ELISA and GC
364 data.

365 Table 2 summarizes the number and percentage of the soil and sediment samples that fall within
366 each of the four categories denoted by whether or not the reported sample concentrations were at or
367 above a specified threshold for either method. If the GC procedure represents a standard method, the
368 false positive rate for the samples was 0% for the SPLE-ELISA method at the comparative levels of
369 1000, 10000, and 100000 ng g^{-1} and increased to 16% at the level of l00 ng g^{-1} level. The false negative
370 rate was 0% at the levels of 1000 and 100000 ng g^{-1} and 3% at the levels of 100 and 10000 ng g^{-1}. Note
371 that the false negative rate at 10000 ng g^{-1} was reduced to 0% if the corrected ELISA data were used.

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

378 The SPLE-ELISA method and the conventional stepwise extraction/cleanup method using either
379 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 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 SPLE-ELISA approach can also be utilized in a tiered approach for the low-cost qualitative screening of samples in human exposure field studies prior to more costly GC Aroclor-specific or GC/MS PCB congener-specific detection methods.

Acknowledgements

The U.S. Environmental Protection Agency through its Office of Research and Development funded and collaborated in the research described here under EPA contract EP-D-04-068 and EP-C-05-057. Some of the work was funded through Battelle's Internal Research and Development Program. We thank all the Battelle staff who carried out sample preparation and analysis activities. This article has been subjected to Agency review and approved for publication. Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

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Figure Caption

Figure 1. Packing of the extraction cell.

Figure 2. Comparison of the corrected SLPE-ELISA Aroclor 1254 EQs and the stepwise extraction/cleanup-GC data summation of Aroclors. 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.

534 **Table 1. Summary Statistics for ELISA Aroclor 1254 EQ and GC/ECD Aroclor Data**

535

Summary Statistics[a]	Uncorrected ELISA Aroclor 1254 EQ, ng g^{-1}	Corrected ELISA Aroclor 1254 EQ, ng g^{-1}	GC Aroclors, ng g^{-1}
Arithmetic Mean	5674	14343	24260
Standard Deviation	24742	70798	128324
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	140625	401786	727250

536 [a] Sample size = 32

537 [b] nd denotes not detected; estimated detection limit was 10 ng g^{-1}.

538

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 %)
10000	0 (0 %)	1 (3 %)	1 (3 %)	30 (94 %)
100000	0 (0 %)	0 (0 %)	1 (3 %)	31 (97 %)

[a] non-corrected ELISA data were used.

544 **Table 3. Summary Statistics for ELISA PCB-126 EQ and GC/MS Coplanar PCB Data**

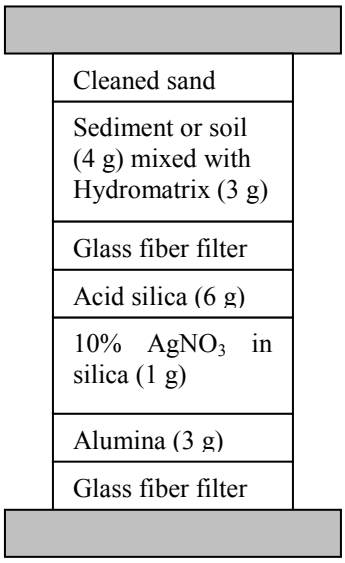
545

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
25^{th} Percentile	4.68	1.27
50^{th} Percentile	15.3	4.01
75^{th} Percentile	53.4	7.73
90^{th} Percentile	94.9	66.0
Maximum	165	116

546 [a] Sample size = 10

547

548 **Figures**

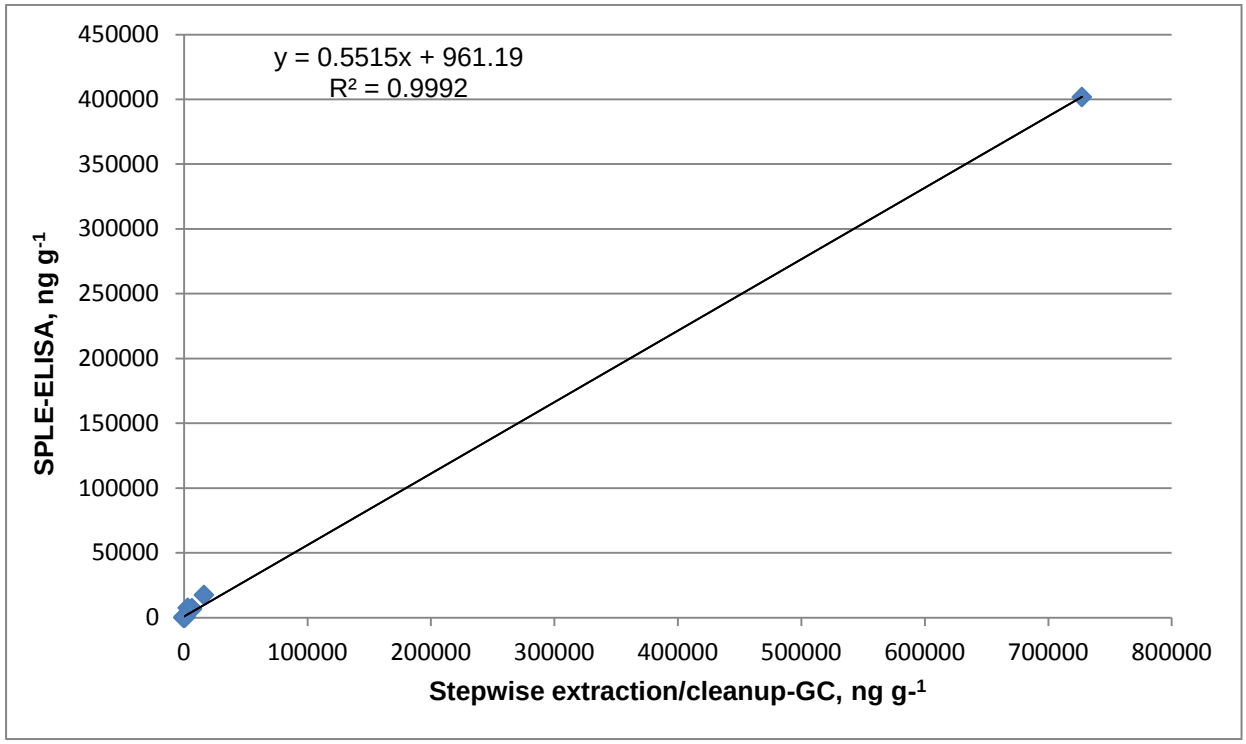


549

550 **Figure 1.**

551

552



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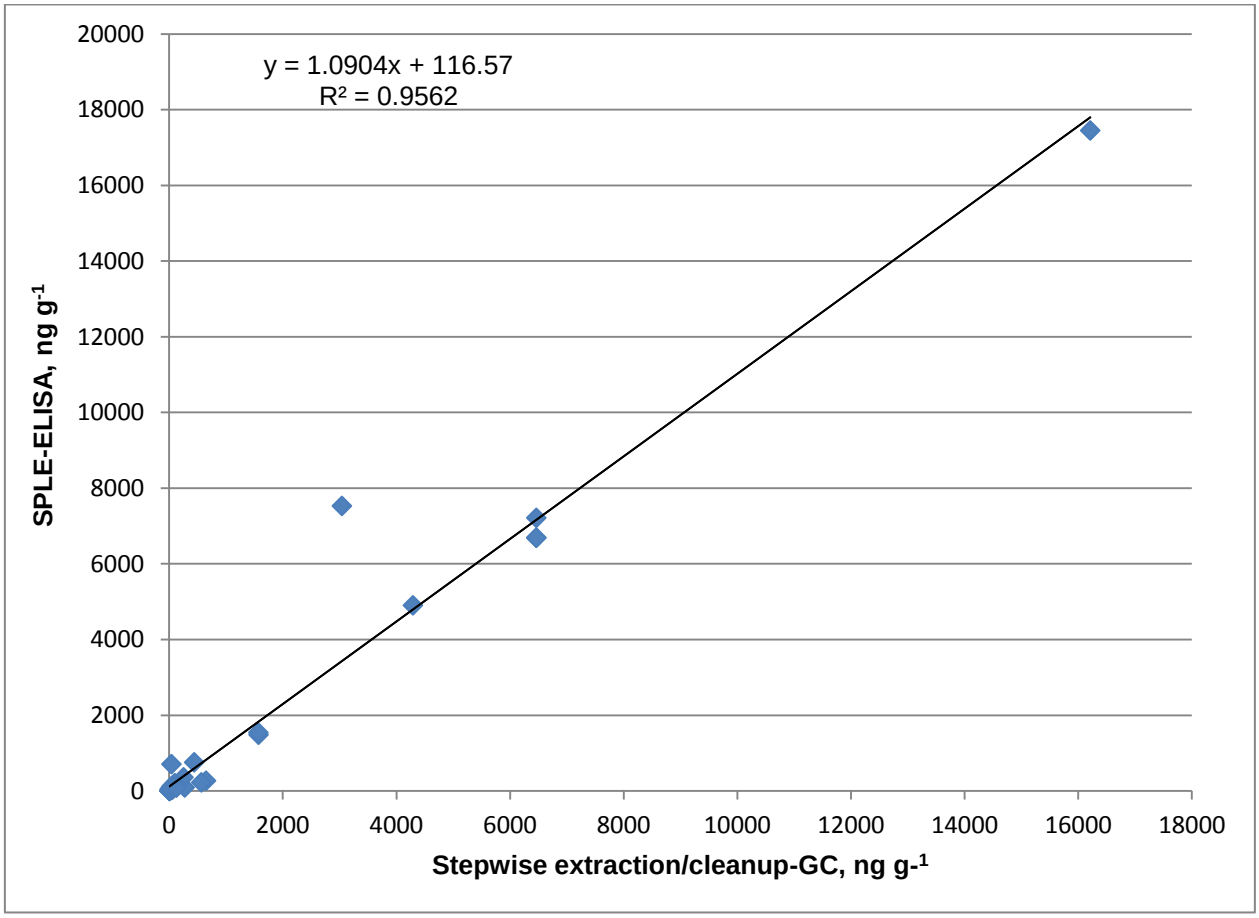

554 **Figure 2**