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Development and Application of Immunoaffinity Chromatography for Coplanar PCBs in Soil and Sediment

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ABSTRACT

An immunoaffinity chromatography (IAC) column was developed as a simple cleanup procedure for preparing environmental samples for analysis of polychlorinated biphenyls (PCBs). Soil and sediment samples were prepared using pressurized liquid extraction (PLE), followed by the IAC cleanup, with detection by an enzyme-linked immunosorbent assay (ELISA). Quantitative recoveries (84-130%) of PCB-126 were obtained in fortified sediment and soil samples using the PLE/IAC/ELISA method. These results demonstrated that the IAC procedure effectively removed interferences from the soil and sediment matrices. The IAC column could be reused more than 20 times with no change in performance

with 99.9% methanol/0.1% Triton X-100 as the elution solvent. Results of 17 soil and sediment samples prepared by PLE/IAC/ELISA correlated well with those obtained from a conventional multi-step cleanup with gas chromatography/mass spectrometry detection.

KEYWORDS: Immunoaffinity chromatography (IAC); coplanar PCBs; soil; sediment; gas chromatography/mass spectrometry; enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Polychlorinated biphenyls (PCBs) are synthetic chemicals that were commonly used as plasticizers, and in capacitors, transformers, and other electrical equipment for insulation. PCBs are a group of 209 different chemicals considered as pollutants of environmental and human health concern. They have been linked to adverse health effects in adults and children (Johnson, et al., 1999; ATSDR, 2000; Aoki, 2001; Schantz, 2003) and are classified as probable human carcinogens by the U.S. Environmental Protection Agency (EPA) (IRIS, 2002). The manufacture of PCBs was banned in the U.S. in 1977 and other countries followed with the Stockholm Convention on Persistent Organic Pollutants in 2001; however, they are still being detected in various environmental components (i.e., air, soil, dust, sediment and food) (Chuang et al. 1998; ATSDR, 2000; Kohler et al. 2002; Wilson et al., 2003; Kim et al., 2004; Sapozhnikova et al., 2004; Hopf, et al., 2009; Chovancova, et al., 2011; Fitzgerald, et al., 2011). Elevated levels of PCBs in building caulking materials from around windows and in expansion joints in masonry buildings have also been reported (Herrick, et al., 2004 and 2007; Van Emon, 2009).

The three non-ortho coplanar PCBs (PCB-77, PCB-126, and PCB-169) are most structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and are considered the most toxic (van den Berg et al., 1998 and

2006). Analytical determination of the coplanar PCBs with conventional methods usually involves an acid wash frequently coupled with either gel permeation chromatography (GPC), or silica/Florisil column chromatography, with gas chromatography/mass spectrometry (GC/MS) or electron capture GC detection (Kohler et al. 2002; Wilson et al., 2003; Kim et al., 2004; Sapozhnikova et al., 2004). Simpler, cost-effective, high-sample throughput cleanup and detection methods may assist in environmental site monitoring and human exposure assessment studies for the PCBs.

IAC combines the advantages of solid phase extraction (SPE) with the specificity of the antibody-antigen (Ab-Ag) interaction. IAC columns have been developed but not employed in large scale for small molecule environmental contaminants (Van Emon et al., 1998; Carrasco et al., 2001; Concejero et al., 2001; Wu et al., 2001; Shelver et al., 2002; Kaware et al., 2006; Altstein, et al., 2007; Chuang et al., 2007). Immunoassay methods have been developed for detecting PCBs at submicrogram levels depending on the congener and the sample processing procedure (Johnson et al., 1996; Van Emon et al., 1992, 2001, 2007; Glass, et al., 2005; Lin, et al., 2008; Tustsumi, et al., 2008; Altstein, et al., 2010).

Described here are: (1) the development of an IAC column with polyclonal rabbit anti-PCB antibodies (Abs) and HiTrap NHS activated Sepharose resin, (2) the development of a PLE method in tandem with an IAC column cleanup and ELISA detection (PLE/IAC/ELISA) and (3) the comparative results generated from different sample preparations (multi-step cleanup, acid wash, and IAC) and detection techniques (GC/MS and ELISA) for coplanar PCB analysis in soil and sediment samples.

EXPERIMENTAL SECTION

Samples. Seventeen sediment and soil samples from various sampling locations in a field study conducted under the U.S. EPA Superfund Innovative Technology Evaluation Monitoring and Measurement Technology program were used for method validation (U.S. EPA, 2004; Dindal et al.,

2007).

Chemicals. Distilled-in-glass grade dichloromethane (DCM), hexane, dimethyl sulfoxide (DMSO), ethyl ether (EE), methanol, toluene, polypropylene glycol (PPG) were from VWR (West Chester, PA). PCB standards were obtained from Cambridge Isotope Laboratories (Andover, MA). Polyclonal anti-PCB Ab (which bound primarily with PCBs 126 and 169) and ELISA testing kits were from Abraxis (Warminster, PA). Glass fiber filters were from Dionex (Sunnyvale, CA). Polymeric Poros resin and silica gel (3-aminopropyl) were purchased from Fisher Scientific (Fair Lawn, NJ). Protein-Pak resin was from Waters (Milford, MA) and Affi-gel 102 was from Bio-Rad Laboratories (Richmond, CA). HiTrap NHS-activated Sepharose (referred hereafter as Sepharose) columns were purchased from Amersham Biosciences (Piscataway, NJ). Non-specific rabbit IgG Ab, bovine serum albumin (BSA), phosphate buffered saline (PBS), PBS containing 0.1% Triton X-100 (PBST), PBS containing 0.1% Tween 20, sulfuric acid, and anhydrous sodium sulfate were obtained from Sigma (St. Louis, MO). Hydromatrix (diatomaceous earth) was purchased from Varian (Walnut Creek, CA).

IAC Development. Five types of control columns were prepared with non-specific rabbit IgG Ab or BSA using (1) Polymeric Poros resin, (2) Protein-Pak resin, (3) Affi-gel 102 (aminoalkyl agarose), (4) silica gel (3-aminopropyl functionalized) and (5) Sepharose resin. Two types of IAC columns were prepared with polyclonal anti-PCB Abs with (1) Affigel and (2) Sepharose. Different combinations of loading solvents (10-25% methanol in water or in PBS) and elution solvents (50-75% methanol in PBS and 100% methanol) were employed. Sepharose resin yielded the best performance results among the five materials tested and was selected for the final development of the IAC procedure. Additional loading solvents evaluated for the Sepharose IAC column were: 1%, 10%, and 25% DMSO in PBST; 1% PPG/20% methanol in PBST; 10% and 20% methanol in PBST; and 10% methanol in PBS with 0.1% Tween 20. In each experiment, the control or IAC column was conditioned with 5 mL of PBS,

followed by 3mL of the loading solvent. After application of a known amount of PCB-126 to the conditioned column, the column was incubated at room temperature for 5 min; washed with 3 or 5 mL of the loading solvent; and eluted with 5 or 10 mL of elution solvent. The elution solvent used for the control column experiments was 100% methanol (1 x 10 mL). Three types of elution solvents were tested for the IAC column: 100% methanol, 99.9% methanol in PBST, and 95% methanol in glycine buffer with 0.1% Triton X-100 (1 x 5 mL or 1 x 10 mL). The flow-through, the wash, and the eluant were analyzed by ELISA. The final optimized IAC procedure is described below for soil and sediment samples.

Extraction of Soil and Sediment. Soil and sediment samples were extracted according to the procedures described in Misita et al., (2003) using a PLE system (ASE 200, Dionex Corp., Sunnyvale, CA, USA) equipped with 33 mL extraction cells. Briefly, an aliquot (10 g) of each sample was mixed with Hydromatrix and extracted with DCM. For fortified samples, a known amount of PCB-126 was spiked onto the soil or sediment prior to extraction. The extractions were performed at 2000 psi at 125^{o}C for 3 cycles of 10 minutes each with a 60% flush. The DCM extracts were then dried with anhydrous sodium sulfate and concentrated to 10 mL. Each sample extract was subjected to various cleanup procedures for either ELISA or GC/MS analysis.

IAC for Soil and Sediment. An aliquot of DCM sample extract was solvent-exchanged into methanol and diluted to 20% methanol in PBST for IAC cleanup. A quality control (QC) solution of PCB-126 (10 ng mL^{-1}) was processed through the IAC column before and after each sample set. The IAC column was conditioned with 5 mL of PBS and 3 mL of the loading solvent (20% methanol in PBST). After applying 1 mL of the QC standard or sample onto the conditioned IAC column, the column was incubated at room temperature for 5 min. The column was then washed with 3 mL of the

loading solvent and the analyte eluted with 3 mL of the elution solvent (99.9% methanol in PBST) in a fraction designated as F1, followed by an additional 2 mL of elution solvent (F2). The IAC column was reconditioned with 5 mL of PBS for subsequent sample loading. A 5 mL aliquot of buffer (0.05 M Na_2HPO_4, 0.1% NaN_3, pH 7) was added after the reconditioning step for column storage.

Acid Wash. An aliquot of DCM sample extract was solvent exchanged into 1 mL of toluene. An aliquot (4 mL) of concentrated sulfuric acid was added to the toluene extract and agitated via a Vortex mixer for 1 min. After the two layers settled, the aqueous layer was discarded and the washing step was repeated until the aqueous layer was colorless. An aliquot (800 µL) of the top layer was then removed, evaporated to dryness under nitrogen, re-dissolved with 1 mL of methanol, and diluted with 1 mL of water (distilled) for ELISA.

Multi-Step Cleanup. Sample extracts for GC/MS analysis were prepared by a multi-step cleanup (Wilson et al., 2003). Briefly, the DCM extracts were concentrated and fractionated by GPC to isolate the PCBs. The target fraction was solvent exchanged into hexane and applied to a Florisil SPE column, preconditioned with 50% EE in hexane and 100% hexane. The fraction that eluted with 15% EE in hexane was concentrated and analyzed by GC/MS.

ELISA Analysis. An aliquot (50 µL) of anti-PCB Ab was first added to each antigen-coated well of a 96-well plate. Next 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 wells and incubated at room temperature for 30 min. An aliquot (50 µL) of the enzyme conjugate solution was then added to each well. The plate was incubated at room temperature for 90

min. The content of the wells were then discarded and the plate was washed with 3 x 250 µL of the washing buffer. After the final wash, an aliquot (150 µL) of the colorimetric enzyme substrate solution was added, followed by an incubation. The absorbance of each well was determined at 450 nm using a Molecular Devices Spectra Max Plus microplate spectrophotometer (Sunnyvale, CA). Data processing was performed with SOFTMaxPro software version 2.1.1.

GC/MS Analysis. A 70 eV electron impact GC/MS (Hewlett-Packard) operated in the selected ion monitoring mode was used. 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 1668A (U.S. EPA, 1999) and followed the overall procedural guidance of EPA Method 8270D (U.S. 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 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 (held for 15 min).

RESULTS AND DISCUSSION

Development of IAC Column. Initial evaluation of the column supporting materials indicated quantitative recoveries were achieved with the Affi-gel (96%) and Sepharose (102%) control columns but not with the Polymeric poros, Protein-PaK, or silica gel columns. Affi-gel and Sepharose were then chosen as resins for the IAC column with 100% methanol as the elution solvent. Quantitative recoveries (72%) of PCB-126 were achieved for the Sepharose IAC column but not for the Affi-gel IAC column (23%). Thus, Sepharose was selected as the support material for the further development of an IAC

column for PCBs.

Average coupling efficiency for the two Sepharose IAC columns was 98±0.5%. The maximum binding capability of the IAC columns was examined by sequential application of PCB-126 to the IAC column until PCB-126 was detected in the flow-through. The results showed that similar maximum loading (~250 ng in 1 mL resin bed) was observed from the two columns and decreasing the amount of methanol in the loading solvent (25% to 10%) did not increase the maximum loading of PCB-126.

Various types of loading solvents were evaluated to minimize the non-specific binding of PCB-126 to the Sepharose. A dilution factor of 200 for the sample containing 0.1% Tween 20 was necessary to remove the high background in the ELISA. Matrix interference from a PPG solvent mix was also observed and a dilution factor of 50 was required prior to ELISA. The Sepharose resin shrunk when exposed to 10% or 25% of DMSO in PBST. These solvents were excluded as loading solvents. ELISA results showed that PCB-126 was not detected in any of the flow through or wash of the Sepharose control column when 10-25% methanol in water or PBS (5 mL) were used as the loading solvents. Recoveries of PCB-126 in the control column flow-through and wash ranged from 88 to 110% when 10%-25% methanol in PBST were used as the loading solvents. These findings suggest that the nonspecific binding was reduced as surfactant was added to the loading solvent. Using these three solvents, PCB-126 was not detected in the flow-through from the IAC column but passed through each control column. These results suggested that the specific binding of PCB-126 to the IAC column is due to the Ab-Ag interaction.

Three elution solvents including 100% methanol, 99.9% methanol in PBST and 95% methanol in glycine buffer with 0.1% Triton were evaluated using the same loading solvent (20% methanol in PBST). Cumulative and quantitative recoveries (96-115%) are shown in Figure 1 for the three elution solvents. Note that the majority of the PCB-126 was eluted in the first 3 mL of the elution solvent and only a residual amount of the PCB-126 was present in the next 2 mL. A neutralization step prior to

188 ELISA was required for the 95% methanol in glycine buffer with 0.1% Triton. Slightly better recoveries
189 of PCB-126 were obtained using 99.9% methanol in PBST as compared with 100% methanol.
190 Column-to column variability was determined using standard solutions applied to the IAC columns
191 and analyzed by ELISA. Quantitative and reproducible recoveries (96±13%) of PCB-126 were obtained
192 from the two IAC columns. PCB-126 was stable in the loading solvent (20% methanol in PBST) at -
193 20^{o}C in the dark for 7 days. These results supported the selection of 20% methanol in PBST and 99.9%
194 methanol in PBST as the loading and elution solvents for processing the real-world soil and sediment
195 samples.

196

197 **PLE/IAC/ELISA for Soil and Sediment.** The Sepharose IAC columns were challenged with 17
198 contaminated soil and sediment samples. The samples were extracted using PLE and the resulting
199 extracts underwent cleanup by IAC and were analyzed by ELISA. Duplicate ELISA analyses were
200 performed and the means of the duplicate values were used to calculate the final concentrations. Data
201 acceptance criteria for the ELISA were established and used as guidance for sample analysis. The four
202 parameter curve-fit values of: (a) upper asymptote, (b) slope, (c) IC_{50}, and (d) lower asymptote were
203 generated for each calibration curve. Figure 2 displays a typical calibration curve for PCB-126. The %
204 relative difference (%D) of the duplicate analyses was within 30% for standard solutions (0.88-29%)
205 and for sample extracts (0.27-29%). Day-to day variation of the ELISA based on 10 standard curves
206 generated on different days, expressed as the % relative standard deviation (RSD) of the IC_{50}, was
207 within ±15% (430±58 pg mL^{-1}). The %D values of the same sample analyzed on different dates were
208 within ±20%. The R^2 value of each calibration curve was greater than 0.99 (0.997±0.003). Recoveries
209 of the back-calculated standard solutions were generally greater than 80% of the expected values. If the
210 ELISA result was outside the calibration range, the sample extract was diluted and re-assayed. Negative
211 control (0 pg mL^{-1}) and positive control (50-500 pg mL^{-1}) standard solutions were also analyzed on each
212 plate. Method blank and negative control sample values were below the assay detection limit (25 pg mL^{-}

213 [1]). Quantitative recoveries ranging from 76 to 113% (average of 93±14%) were obtained for the positive
214 controls. The overall assay precision was within ±30% and the overall accuracy for PCB-126 was
215 greater than 70%, which are comparable with results obtained by GC/MS analysis (typically precision
216 within ±20% and accuracy >80%).

217 The precision and accuracy of the PLE/IAC/ELISA method were evaluated with real world soil and
218 sediment samples previously determined to contain PCBs (unpublished data). The samples were spiked
219 with PCB-126 at 2, 5, and 10 ng g^{-1} and both the nonspiked and spiked samples were processed through
220 the PLE/IAC/ELISA. Quantitative recoveries of PCB-126 were achieved in the spiked samples
221 (108±21%) by the PLE/IAC/ELISA method. Quantitative recoveries of PCB-126 were also obtained in
222 the post-spiked PLE sample extracts (103±16%) using IAC cleanup followed by ELISA. The %D
223 values of the same sample extract from two different dilutions, within the assay calibration range, were
224 less than 20% (0.16-17%). These findings demonstrated that the IAC was an effective alternative
225 cleanup procedure in removing interference components from the soil and sediment samples.
226 Recoveries of the QC standard (10 ng mL^{-1} of PCB-126) processed through the IAC column before and
227 after processing each set of field samples ranged from 82 to 113% indicating that the IAC column was
228 functioning properly after processing several real-world samples. In summary, overall method precision
229 (PLE/IAC/ELISA) was within ±20% and the overall recovery for PCB-126 in soil and sediment was
230 greater than 80%.
231

232 **Analytical Methods Comparison.** Different aliquots of 17 soil and sediment samples were prepared
233 and analyzed by three different analytical methods: (1) PLE/multi-step cleanup/GC/MS, (2) PLE/acid
234 wash/ELISA, and (3) PLE/IAC/ELISA. The same PLE extraction conditions were used in the three
235 analytical methods. Sample matrix interference was observed for the soil and sediment sample extracts
236 without any cleanup procedures by either GC/MS or ELISA detection. A multi-step cleanup procedure

was required in order to achieve quantitative recoveries (86-135% of the spiked coplanar PCBs in soil and sediment samples) by GC/MS. The matrix interference for ELISA was removed by either IAC or an acid wash but repeated acid wash steps were required for some samples. Recoveries of PCB-126 determined by ELISA in the matrix spiked soil and sediment samples/sample extracts ranged from 68 to 147% by acid wash and from 84-130% by IAC. Among the three cleanup methods, the IAC procedure is the least labor-intensive and provides the highest sample throughput. The most complicated and time-consuming procedure is the multi-step cleanup required for the GC/MS analysis.

The two detection techniques (ELISA and GC/MS) utilize different principles in determining coplanar PCBs. The ELISA was calibrated against PCB-126 and provided a single measurement representing the PCB-126 equivalent (EQ) value in a given real-world sample. This value accounts for the levels of other PCB congeners that respond to the Ab due to cross reactivity (CR). CRs 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 ELISA had very low CRs to Aroclors (<0.1%). In contrast, the GC/MS-derived results provided specific measured concentrations for each of the 12 coplanar PCBs. The ELISA-derived PCB-126 EQ values were compared with the sums of 12 coplanar PCBs derived by GC/MS for determination of the PLE/IAC/ELISA as a screening method.

Summary statistics for the soil and sediment samples analyzed by the three analytical methods are shown in Table 1. A wide concentration range was observed in the 17 soil and sediment samples. The highest coplanar PCB concentration as determined by all three methods was found in the soil sample taken from a PCB landfill site. In general, the ELISA-derived PCB-126 EQ concentrations were similar to or higher than the sums of the 12 coplanar PCBs by GC/MS. Similar ELISA-derived PCB-126 EQ values were obtained in most samples using two different cleanup procedures (acid wash and IAC). The higher ELISA-derived PCB-126 EQ data could be due to the CR for other PCBs and/or PCB-like

compounds that were not measured by GC/MS but have a high likelihood of being present in the samples. ELISA-derived PCB-126 EQ values and GC/MS-derived sums of 12 coplanar PCBs for all the samples were highly correlated, with a correlation coefficient of 0.99.

An effective screening method is expected to have zero false negative and low false positive rates when compared with an established standardized method. Table 2 summarizes the false positive, false negative, true positive, and true negative rates of the PLE/IAC/ELISA method. The measurements derived from the PLE/multi-step cleanup/GC/MS were treated as reference values and the ELISA-derived PCB-126 EQ values were compared with the sums of 12 coplanar PCBs at four concentration levels (1, 10, 100, and 1000 ng g^{-1}). The false negative rates were 0% at the four comparative levels for all samples (N = 17). The false positive rates were 0% at 1 and 1000 ng g^{-1} and 6% at 10 and 100 ng g^{-1}.

CONCLUSIONS

Coplanar PCB IAC columns were made by immobilizing anti-PCB antibodies onto a Sepharose column. The optimized loading and elution solvent systems for the IAC column were 20% methanol in PBST and 99.9% methanol in PBST, respectively. The coupling efficiency for the IAC columns was 98% and the maximum loading of PCB-126 for the IAC columns was approximately 250 ng of PCB-126 in 1 mL of resin bed (4.8 mg of Ab). The IAC columns are robust and can be regenerated and reused for multiple samples in a routine laboratory operation. The binding efficiency did not decrease after processing more than 20 spiked and non-spiked soil and sediment sample extracts and numerous standard solutions.

Coupling PLE with immunochemical cleanup and detection methods provided a new tandem approach (PLE/IAC/ELISA) for monitoring coplanar PCBs. Quantitative recoveries (84-130%) of PCB-126 were achieved in the fortified soil and sediment samples. The ELISA-derived PCB-126 EQ levels correlated

well, but were generally higher than the GC/MS-derived sums of 12 coplanar PCBs. The 0% false negative rate and low false positive rate (0% or 6% depending on the threshold level) observed in the 17 environmental samples indicate that the PLE/IAC/ELISA can be an effective screening method for coplanar PCBs in soil and sediment.

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297 **REFERENCES**

298 Altstein, M., Bronshtein, A. 2007. Sol-gel immunoassays and immunoaffinity chromatography. In:
299 Van Emon J.M. ed. Immunoassay and Other Bioanalytical Techniques, CRC Press, Taylor and
300 Francis, New York, pp. 357-383.

301 Altstein, M., Aziz1, O.B., Skalka, N., Bronshtein1, A., Chuang, J.C., Van Emon, J.M. 2010.
302 Development of an immunoassay and a sol-gel based immunoaffinity cleanup method for
303 coplanar PCBs from soil and sediment samples, Anal. Chim. Acta. 675, 138-147.

304 ATSDR, 2000. (Agency for Toxic Substances and Diseases Registry). Toxicological

305 Profile for Popychlorinated Biphenyls (PCBs), http://www.atsdr.cdc.gov/ToxProfiles/tp17.pdf , U.S.
306 Department of Health and Human Service, Atlanta, GA.

307 Aoki, Y. 2001. Polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, and polychlorinated
308 dibenzofurans as endocrine disrupters—what we have learned from Yusho disease, Environ.
309 Res. 86, 2-11.

310 Carrasco, P.B., Escola, R., Marco, M.P., Bayona, J.M. 2001. Development and application of
311 immunoaffinity chromatography for the determination of the triazinic biocides in seawater, J.
312 Chromatogr. A. 909, 61-72.

313 Chovancova, J., Conka, K.,Kocan, A., Sejakova, Z.S. 2011. PCDD, PCDF, PCB, and PBDE
314 concentrations in breast milk of mothers residing in selected area of Solvakia, Chemosphere. 83,
315 1383-1390.

316 Chuang, J.C., Miller, L.S., Davis, D.B., Peven, C.S., Johnson, J.C., Van Emon, J.M. 1998. Analysis of
317 soil and dust samples for polychlorinated biphenyls by enzyme-linked immunosorbent assay
318 (ELISA). Anal. Chim. Acta. 376, 67-75.

Concejero, M.A., Galve, R. , Herradon, B. , Gonzalez, M. , de Frutos, M. 2001. Feasibility of high-performance immunochromatography as an isolation method for PCBs and other dioxin-like compounds, Anal. Chem. 73, 3119-3125.

Dindal, A., Thompson, E., Aume, L., Billets, S. 2007. Application of site-specific calibration data using the CALUX by XDS bioassay for dioxin-like chemicals in soil and sediment samples. Environ. Sci. Technol. 41, 8376-8382.

Fitzgerald, E.F., Shrestha, S., Palmer, P.M., Wilson, L.R., Belanger, E.E., Gomez, M.I., Cayo, M.R., Hwang, S.A. 2011. Polychlorinated biphenyls (PCBs) in indoor air and in serum among older residences among upper Hudson River community, Chemosphere. 85,225-231.

Glass, T.R., Ohmura, N., Taemi, Y., Jon, T. 2005. Simple immunoassay for detection of PCBs in transformer oil, Environ. Sci. Technol. 39, 5005-5009.

Herrick, R.F., McClean, M.D., Meeker, J.D., Baxter, L.K., Weymouth, G.A. 2004. An unrecognized source of PCB contamination in schools and other buildings. Environ. Health Persp. 112 (10), 1051-1053.

Herrick, R.F., Lefkowitz, D.J., Weymouth, G.A. 2007. Soil contamination from PCB-containing buildings. Environ. Health Persp. 115 (2), 173-175.

Hopf, N.B., Ruder, A.M., Succop, P. 2009. Background levels of polychlorinated biphenyls in the U.S. population, Sci. Total Environ. 407, 6109-6119.

IRIS (2002). Polychlorinated biphenyls (PCBs) (cancer assessment last updated 6/01/1997). Integrated Risk Information System. U.S. Environmental Protection Agency. http://www.epa.gov/iris/subst/0294.htm

Johnson, B.L., Hicks, H.E., Cibulas, W., Faroon, O., Ashizawa, A.E., DeRosa, C.T. 1999. Public

341 health implications of exposure to polychlorinated biphenyls (PCBs). Agency for Toxic
342 Substances and Disease Registry. http://www.atsdr.cdc.gov/DT/pcb007.html

343 Johnson, J.C., Van Emon, J.M. 1996. Quantitative enzyme-linked immunosorbent assay for
344 determination of polychlorinated biphenyls in environmental soil and sediment samples, Anal.
345 Chem. 68, 162-169.

346 Kaware, M., Bronshtein, A., Safi1, J., Van Emon, J.M., Chuang, J.C., Hock, B., Kramer, K., Altstein,
347 M. 2006. Enzyme-linked immunosorbent assay (ELISA) and sol-gel based immunoaffinity
348 purification (IAP) of the pyrethroid bioallethrin in food and environmental samples. J. Agric.
349 Food Chem. 54, 6482–6492.

350 Kim, M., Kim, S., Yun, S., Lee, M., Cho, B., Park, J., Son, S., Kim, O. 2004. Comparison of seven
351 indicator PCBs and three coplanar PCBs in beef, pork, and chicken fat, Chemosphere. 54, 1533-
352 1538.

353 Kohler, M., Zennegg, M., Waeber, R. 2002. Coplanar polychlorinated biphenyls (PCB) in indoor air,
354 Environ. Sci. Technol. 36, 4735-4740.

355 Lin, Y.Y., Liu, G., Wai, C.M., Lin, Y. 2008. Bioelectrochemical immunoassay of polychlorinated
356 biphenyls. Anal. Chim. Acta. 612, 23–28.

357 Misita, M., Schrock, M., Tracy, K., Tabor, J. 2003. Simultaneous extraction of PCDD/PCDF and PCBs
358 using accelerated solvent extraction for sediment, tissue, and sludge matrices. Organohalogen
359 Compounds. 60, 37-40.

360 Sapozhnikova, Y., Bawardi, O., Schlenk, D. 2004. Pesticides and PCBs in sediments and fish from the
361 Salton Sea, California, USA, Chemosphere. 55, 797-809.

362 Schantz, S.L., Widholm, J.J., Rice, D.C. 2003. Effects of PCB exposure on neuropsychological

function in children, Environ. Health Persp. 111, 357-576.

Shelver, W.L., Shan, G., Gee, S.J., Stanker, L.H., Hammock, B.D. 2002. Comparison of immunoaffinity column recovery patterns of polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans on columns generated with different monoclonal antibody clones and polyclonal antibodies, Anal. Chim. Acta. 457, 199-209.

Tsutsumi, T., Miyoshi, N., Sasaki, K., Maitani, T., 2008. Biosensor immunoassay for the screening of dioxin-like polychlorinated biphenyls in retail fish. Anal. Chim. Acta. 617, 177-183.

U.S. EPA, 1999. Method 1668, Revision A, Chlorinated Biphenyl Congers in Water, Soil, Sediment, and Tissue by HRGC/HRMS, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 2004: Demonstration and Quality Assurance Project Plan Technologies for the Monitoring and Measurement of Dioxin and Dioxin-Like Compounds in Soil and Sediment, EPA/600/R-04/036.

U.S. EPA, 2006. Method 8270D, Revision 4, Semi-volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), U.S. Environmental Protection Agency, Washington, D.C.

Van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunström, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasagawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., Van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Env. Health Persp. 106, 775-792.

Van den Berg, M., Birnbaum, L., Dension, M., DeVito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Scherenk, D., Tohyama, C., Tritscher,

A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R. E., 2006. Human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds: The WHO 2005 re-evaluation. Toxicol. Sci. 93(2), 223-241.

Van Emon, J.M., Lopez-Avila, V. 1992. Immunochemical methods for environmental analysis. Anal. Chem. 64, 79A-88A.

Van Emon, J.M., Gerlach, C.L., Bowman, K. 1998. Bioseparation and bioanalytical techniques in environmental monitoring. J. Chromatogr. B. Biomed Sci. Appl. 715, 211-228.

Van Emon, J.M. 2001. Immunochemical applications in environmental science. J. of AOAC Int. 84, 125-133.

Van Emon, J.M., Chuang, J.C., Trejo, R.M., Durnford, J., 2007. Integrating bioanalytical capability in an environmental analytical laboratory. In: Van Emon, J.M. Ed. Immunoassay and Other Bioanalytical Techniques, CRC Press, Taylor and Francis, New York, pp 1-43.

Van Emon, J.M. Application of Rapid Field Screening Inexpensive Immunoassays for the Assessment of Buildings Contaminated with PCBs, U.S. EPA Region II RARE Project Report, Las Vegas, NV, April 2009.

Wilson, N.K., Chuang, J.C., Lyu, C.W., Menton, R., Morgan, M. 2003. Aggregate Exposures of Nine Preschool Children to Persistent Organic Pollutants at Day Care and at Home, J. Expo. Anal. Environ. Epidemiol. 13, 187-202.

Wu, Z., Junsuo, L., Zhu, L., Luo, H., Xu, X. 2001. Multi-residue analysis of avermectins in swine liver by immunoaffinity extraction and liquid chromatography-mass spectrometry, J. Chromatog. B. 755, 361-366.