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Evaluation of Immunoassay Tests in Screening Soil Contaminated with Polychlorinated Biphenyls

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Many field remediations require the analysis of a tremendous number of samples to determine the extent of contamination and effectiveness of remediation. Gas chromatographic (GC) analysis is generally employed for this purpose. However, it is costly, ranging from $100-$400 per sample, and has a turnaround time of 1-30 days. Significant cost savings could be realized with fast and inexpensive sample analysis.

Immunoassay is based on antibody-antigen reactions that are specific and occur quickly. This technique has been used successfully in clinical diagnostics for many years. Different antibodies have been developed for many chemicals, particularly pesticides [Jung et al. 1989; Clower 1991] and toxic chemicals [Schwalbe-Fehl 1986]. Two companies have developed polychlorinated biphenyls (PCBs) soil screening kits using the competition enzyme-linked immunosorbent assay (cELISA). Recently, EPA approved the PCB-RISc™ of EnSys, Inc., as a field analytical for hazardous site assessment (EPA Method 4020). Because of specificity and fast reaction, this technique was adopted quickly in the environmental remediation field [Van Emon 1990; Van Emon and Lopez-Avila 1992]. A sample containing PCBs is introduced to a test tube of immobilized antibody. PCBs bind to antibody sites. The remainder of the antibody sites are occupied by PCB conjugates. In the presence of the conjugates (horseradish peroxidase), a substrate (hydrogen peroxide) oxidizes the chromogen (tetramethylbenzidine) and causes it to change from colorless to blue. Therefore, color intensity is inversely proportional to PCB concentration. The binding process is a highly specific interaction between a target compound and an antibody through geometric fitting, hydrophobic interaction, ionic interaction, etc. [Stanker et al. 1990]. Because of this specificity, the immunoassay test of triazine herbicides in water was found to be well correlated with GC/MS analysis [Thurman et al. 1990].

The objective of this study was to evaluate two brands of PCB soil immunoassay kits for a valid field screening tool. The two kits were PCB-RISc™ marketed by EnSys, Inc., Morrisville, North Carolina, and EnviroGard™ (ENVR 000 10) marketed by Millipore Corp., Bedford, Massachusetts. Validity of these tests was assessed by three criteria: precision (reproducibility), accuracy, and cross reactivity.

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MATERIALS AND METHODS

Backyard soil which had a moisture content of 12.7% and organic matter of 5% was used in this study. Large-sized gravel and plant debris were removed from the soil, and soil aggregates were crushed by hand. Nonaqueous phase liquid (NAPL), containing many chlorinated aliphatics, chlorinated aromatics, and PCBs, was used in making soil samples for immunoassay tests. Instead of pure PCB solution, the NAPL obtained from contaminated groundwater was used to examine the interferences of other compounds to the PCB immunoassay. The PCBs of the NAPL were classified as a weathered Aroclor 1248. Aroclor 1248 consisted of dichlorobiphenyls (2%), trichlorobiphenyls (18%), tetrachlorobiphenyls (40%), pentachlorobiphenyls (36%), and hexachlorobiphenyls (4%). Numbers in parentheses are approximate weight percents.

Various amounts of two NAPLs were dissolved in 50 mL of acetone. NAPL "A" contained 2.4 ppm of PCBs and was used to prepare a soil sample with a negligible amount of PCBs (Soil-A). Soil-A, however, had a high level of chlorinated aliphatics (1%). NAPL "B" which contained 3,250 ppm of PCBs was used for preparing four other spiked soils at PCB concentrations higher than 1 ppm (Soil-B, Soil-C, Soil-D, Soil-E). Each acetone solution was mixed with 80 g of the soil in a 120-mL glass bottle for 3 hr. Sample bottles were kept uncapped in a hood for a day to allow the acetone to volatilize. Then, the soil samples were disaggregated and tumbled once a day for a week. An unspiked soil was analyzed by gas chromatography (GC) and mass spectrometry (MS), following modified EPA Method 8270A. PCBs and chlorinated aliphatics/aromatics were not detected in the unspiked soil. One soil sample was prepared as a blank in the same way with pure acetone but without the addition of the NAPLs (Blank).

Other soil samples were collected from a site contaminated with Aroclor 1248 to compare with the spiked soils. Aroclor 1248 concentrations of the site soils were less than 100 ppm, as determined by EPA Method 8080 (GC/ECD). The site soil was sandy and moist by physical inspection. Following procedures of field immunoassay tests, these samples were not sifted but prepared only by removing plant debris and large-sized gravel.

Two grams of each soil sample were extracted by vigorous mixing with 2 mL of dimethyl sulfoxide (DMSO) in a plastic syringe packaged in the test kit. A filter was attached to the syringe to collect 5 μL of the DMSO extract. The test was conducted with one negative control and two calibrators (7 and 45 ppm). PCBs in the DMSO extracts and in the calibrators bound to antibodies on the test tube wall during a 5-min incubation period. The test tube content was removed by intense squirting with distilled water. PCB-HRP conjugates were allowed to react with the remainder of the antibody sites for the next 5 min. Free conjugates were washed out by distilled water. Substrate and chromogen solution were added to the tubes in sequence. In the presence of bound conjugates, the substrate was converted to a compound which causes the chromogen to turn blue. Sulfuric acid was added to stop further color development, and the color then became yellow. Color intensity was measured at 450 nm by using a spectrophotometer (Model DR/3, HACH Company, Loveland, Colorado) for quantitative comparison. Consequently, the manufacturer's of qualitative measurement was slightly modified by adding 3 mL of distilled water in tube samples. This addition of distilled water diluted the color intensity, but relative comparisons in the differences of sample's color intensity still were possible.

Millipore Corp. later changed the extractant from DMSO to methanol and adopted the use of bead mixing for soil extraction. Only this new EnviroGard kit was available when the site soils were tested. Five milliliters of methanol were used to extract PCBs from five grams of the soil. The extraction was achieved by vigorous agitation with five stainless steel beads. The extracts were filtered through a plastic syringe filter (Autovial Syringeless Filter, 0.45 μm, Genex Corp., Gaithersburg, Maryland). Three calibrators (5, 10, and 50 ppm) were employed. The new test kit required the use of a pipet, instead of a dropper, to deliver a volume of reagents precisely. The rest of the procedure was the same as described above.

Ten grams of each spiked soil was extracted by mixing with 20 mL of methanol in a plastic bottle with five stainless steel beads (8 mm dia). Consequently, the PCB concentration in the soil was reduced at least by half in the soil extract. Thirty microliters (30 μL) of methanol extract were collected through the plastic syringe filter. The test required fivefold dilution for the 5-ppm test and an additional tenfold dilution for the 50-ppm test. All the dilution was performed in dilution vials that contained a required volume of methanol. This test used one calibrator which was labeled as 0.5 ppm, but it was reportedly 0.3 ppm. This concentration was chosen by the manufacturer to provide 40% safety over 0.5 ppm. This means that the test was biased toward false positive which was considered safer than false negative. If the color intensity of the first diluted sample is less than that of the calibrator, the sample contained more than 5 ppm of PCBs. For the second diluted sample, it meant the sample contained more than 50 ppm of PCBs. The principles of the PCB-RISc test were the same as previously described for the EnviroGard test.

GC/MS analysis of PCBs was employed for the soil samples of the PCB-RISc test and their methanol extracts. Five grams of the soil sample were Soxhlet extracted using methylene chloride/hexane (1:1). The extract then was concentrated into 100% hexane. Alumina cleanup was performed on the hexane concentrates prior to the analysis by following EPA Method 3610A. The analysis showed that the spiked soils contained trichlorobiphenyls, tetrachlorobiphenyls, and pentachlorobiphenyls more than the detection limit of 0.1 ppm among chlorinated biphenyls. A surrogate (3,3',4,4'-tetrachlorobiphenyl) was added to the soils, and surrogate recovery was in the range of 41 to 86%. An average moisture content of the soil samples was 15%. PCB concentrations in the soils were corrected for an average surrogate recovery of 59.4% and reported on the dry weight basis. The methanol extracts of the PCB-RISc test were dissolved in distilled water, extracted with methylene chloride three times, followed by solvent exchange with hexane. The hexane sample was concentrated by evaporation. The hexane concentrates were analyzed by using a GC/MS. The surrogate (3,3',4,4'-tetrachlorobiphenyl) recovery of the methanol extract analysis ranged from 86 to 107%. Correction for an average surrogate recovery of 99.2% was applied to PCB concentrations in the methanol extracts.

It was found that the recovery of DMSO extracts was unacceptably low with the GC/MS analytical method. Therefore, DMSO extracts and corresponding soils of the EnviroGard test were analyzed by using a GC with an electron capture detector (ECD) for Aroclor 1248. PCBs in the NAPL most resembled Aroclor 1248. Following EPA Method 3550, soil was extracted three times by sonication with either acetone/methylene chloride (1:1) or hexane, depending on PCB concentration. The extracts were concentrated in a Kuderna-Danish apparatus.

The concentrates were analyzed by a GC/ECD. A surrogate (Aroclor 1248) was added to one of the soil samples, and an average surrogate recovery was 88%. The average moisture content of the soil samples was 15%. PCB concentrations in the soils were corrected for the surrogate recovery and reported on the dry weight basis. Because a broad DMSO peak interfered with Aroclor analysis, one part of the DMSO extract was diluted with three parts of acetone. The diluted extracts then were analyzed by a GC/ECD with EPA Method 8080. An average surrogate (Aroclor 1248) recovery of the DMSO extract analysis was 115%. The results were corrected for the recovery. The method detection limit of the GC/ECD was 0.1 ppm.

EPA Method 8080 with a simplified extraction was employed in the field for the PCB analysis of the site soil. Two grams of soil were extracted with hexane by vigorous shaking in a bottle. PCB concentrations in the hexane extracts were measured by a GC/ECD in the field. The surrogate (Aroclor 1248) recovery of this analysis was 86%, and PCB concentrations in the soils were corrected for that. The detection limit of this field analysis was 5 ppm.

Table 1. PCB concentrations were compared with immunoassay results of the EnviroGard test (Millipore Corp.).

Sample	PCB Concentration, ppm		Transmittance, %	Immunoassay Result, ppm
	In Soil	In DMSO Extract		
Blank	< 0.1	< 0.1	65, 65	< 7
Soil-A	< 0.1	0.2	68, 74	< 7
Soil-B	1.4	0.3	72, 70	< 7
Soil-C	11.8	2.0	75, 75	< 7
Soil-D	21.2	6.1	84, 83	7 - 45
Soil-E	107.5	34.9	90, 90	7 - 45

PCB concentrations in the soils and in the DMSO extracts were measured separately. Average transmittances of three standards in four replicates with 95% confidence interval: 0 ppm = 52.3% ± 4.4%; 7 ppm = 82.0% ± 5.2% ; 45 ppm = 94.5% ± 4.2%

RESULTS AND DISCUSSION

Accuracy and cross reactivity are two important criteria in selecting an immunoassay technique to screen contaminated soils. Immunoassay tests were evaluated by using soil spiked with NAPL containing PCBs. Because a low level of PCBs is present with a high level of other chemicals in many contaminated sites, it is important to examine cross reactivity of the immunoassay tests. Constituents of the NAPL were mostly chlorinated aliphatics and aromatics, including hexachlorobenzene (C_6Cl_6). Concentrations of the NAPL in the spiked soils ranged from approximately 0.01 to 1%. Cross reactivity of various compounds and mixtures had been investigated by the immunoassay manufacturers. Generally, cross reactivity increased as the structure of reactants became similar to PCBs, such as pentachlorobenzene [Mapes et al. 1992]. A high concentration of cross-reacting agents or other substances, such as oil, may

hinder PCB extraction from soil by either saturating an extractant or blocking contact with an extractant [Harrison and Charmerlik-Cooper 1992]. The Blank soil in Tables 1 and 2 had no spiking of PCBs. The other soil samples were spiked at different levels of PCB concentrations less than 200 ppm. Soil-A in Tables 1 and 2 contained almost no PCBs but a high concentration (1%) of other chlorinated compounds. Using these spiked soils, the accuracy of the immunoassay results was examined through comparison with the GC results.

Table 2. PCB concentrations were compared with immunoassay results of the PCB-RISc test (EnSys Inc.).

Sample	PCB Concentration, ppm		Transmittance, %			Immunoassay Result, ppm		
	In Soil	In Methanol Extract	Sample in Dilution, %			Sample in Dilution, %		
			100	10	1	100	10	1
Blank	< 0.1	< 0.2	40	34	32	< 0.5	< 5	< 50
Soil-A	< 0.1	< 0.2	39	29	29	< 0.5	< 5	< 50
Soil-B	2.4	0.2	44	38	31	0.5	< 5	< 50
Soil-C	12.8	2.3	57	58	43	> 0.5	> 5	< 50
Soil-D	25.8	8.6	59	48	40	> 0.5	> 5	< 50
Soil-E	182.9	34.9	62	56	61	> 0.5	> 5	> 50

PCB concentrations in the soils and in the methanol extracts were measured separately. The extract concentrations were corrected for 1:2 dilution. Average transmittances of two standards in five replicates with 95% confidence interval: 0 ppm = 37.6% ± 1.3% ; 0.5 ppm = 44.0% ± 1.2%

Color intensity of immunoassay samples was measured at 450 nm by a spectrophotometer in % transmittance. Transmittance was proportional to a PCB concentration in samples. PCBs in samples competed for antibody sites with conjugates which were involved in color development. There was no significant difference in transmittance between the blank soil and Soil-A (<0.1 ppm PCB) (Tables 1 and 2). Soil-A containing 0.5% tetrachloroethylene, 0.5% trichloroethylene, and other chlorinated compounds demonstrated no response to PCB immunoassay, indicating that cross reactivity of these compounds was minimal. The precision of this method was evaluated by measuring transmittances of the negative and the calibrators in replicates (4 for EnviroGard and 5 for PCB-RISc): % transmittance ranges of EnviroGard's negative, 7 ppm calibrator, and 45 ppm calibrator were 50-55, 79-85, and 92-97, respectively; % transmittance ranges of PCB-RISc' control and standard were 36-39 and 42-45, respectively. Precision (reproducibility) of both tests was good in comparison with the quality control acceptance criteria of EPA Methods.

One distinct difference between the two immunoassay tests was sample preparation. The PCB-RISc test required sample dilution but the EnviroGard test did not. In the PCB-RISc test, diluted samples were compared to one calibrator. The sample dilution was incorporated in the PCB-RISc test because immunoassay exhibited a high sensitivity in the low range of PCB concentrations. The

immunoassay test is analogous to carbon adsorption which has a saturation point. Adsorption approaches the saturation point asymptotically, meaning that linearity lies at a low concentration range. Sensitivity is greater in this range than in the higher range. On the other hand, the EnviroGard test used two calibrators (7 and 45 ppm) without sample dilution. As shown in Table 2, more diluted samples had transmittance higher than less diluted samples, suggesting that dilution might have caused errors in the PCB-RISc test. Recently the EnviroGard test changed this calibration scheme from two points to three points (5, 10, and 50 ppm). This scheme provides the test to screen soils closely and was used in the test of the site soil.

Another difference between the two tests was the organic solvent for extracting PCBs from soil. The EnviroGard used DMSO which was a strong polar solvent. But extraction was performed in an inefficient way. A soil sample and DMSO were mixed in a small plastic syringe provided in the test kit. DMSO extraction efficiency ranged between 17 and 33% by comparing PCB concentrations in the soils with those in the extracts (Table 1). The EnviroGard test previously employed soil sampling by volume. An amount of soil would vary significantly depending on soil moisture content, soil structure, etc. Recently the EnviroGard test adopted the same extraction scheme as the PCB-RISc test, except 5 g of soil was extracted by using 5 mL of methanol. The PCB-RISc test used methanol to extract PCBs from soil. A large amount of soil (10 g) was involved in the extraction for better sample representativeness. In order to increase sample mixing, five stainless steel beads (8 mm dia) also were included in a 50-mL plastic bottle containing 20 mL of methanol. Despite this more elaborate mixing arrangement, methanol extracted only an average 20% of PCBs with the range of 9 to 33% (Table 2). The extraction efficiency of both tests needs to be improved in order to screen soil effectively in the field.

Table 3. PCB concentrations of the site soil were compared with immunoassay results of the EnviroGard test (Millipore Corp.).

PCB Conc. in Soil, ppm	Transmittance, %	Immunoassay Result, ppm
< 5	60	< 5
< 5	48	< 5
< 5	61	5
5.8	68	10 - 50
24.4	69	10 - 50
40.7	80	>50
59.3	74	10 - 50
69.8	75	10 - 50

Wet weight basis was used in the measurement of PCB concentrations in the soils and the immunoassay. Transmittance data above are averages of duplicates. Transmittances of three standards: 0 ppm = 36% ; 5 ppm = 61% ; 10 ppm = 63% ; 50 ppm = 76%

Two false negatives (33% tested) were detected in the EnviroGard test (Table 1). In comparison to the calibrators, 11.8 ppm soil was screened at less than 7 ppm, and 107.5 ppm soil was screened at less than 45 ppm. However, one false positive (16.7% tested) was found when the immunoassay results were compared to PCB concentrations in the DMSO extracts (Table 1). Acknowledging that a false positive is safer than a false negative, this test could be a reliable screening tool if extraction efficiency was 100%, or consistent and then adjusted accordingly. Only a small difference in the transmittances of duplicate assays indicated good reproducibility of this test in which an aliquot of the same DMSO extract was used. No false readings against PCB concentrations in the soils were detected in the PCB-RISc test but two false positives (33.3% tested) were found in comparison with PCB concentrations in the methanol extracts (Table 2). The extract concentration of 2.3 ppm was screened at more than 5 ppm and 34.9 ppm at more than 50 ppm. These false positive readings could be attributed to the fact that a built-in safety factor was too conservative. EnSys intentionally took this conservative approach to minimize false negative readings.

The site soil was tested with the new EnviroGard kits which used methanol, bead mixing, three-point calibration, and pipetting. The site soil contained Aroclor 1248 at less than 70 ppm measured by a GC/ECD. Three false positives (37.5% tested) and two false negatives (25% tested) were recorded (Table 3). Despite some recent modification, the EnviroGard test still exhibited a significant number of false readings. This could be due to ineffective methanol extraction and/or insufficient soil preparation. Of course, a number of false readings can be reduced by incorporating a safety factor as in the PCB-RISc test.

Overall, the specificity of the immunoassay was high with a low cross reactivity, but the EnviroGard test had some false readings. Immunoassay can be used for reliably screening complex environmental samples which contain multiple components. However, the usefulness of these test kits was limited by inappropriate consideration of some factors. Soil extraction efficiency must be taken into account in the EnviroGard test; otherwise, serious errors will occur because of false negative readings. A safety factor built in the PCB-RISc tests appeared to be too conservative, potentially resulting in unnecessary remedial efforts. Some false readings also could be avoided by homogenizing soil samples and extracting PCBs effectively. The immunoassay test kits are inexpensive (<$20 per sample) as compared to traditional GC analysis. The test for 3-4 samples, including extraction, took less than one hour. The fast turnaround time can shorten remediation time and save operating costs.

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