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Penta RISc™ Soil—A Rapid, On-Site Screening Test for Pentachlorophenol in Soil

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Currently, environmental engineers and consultants must often wait days to receive reliable test results on soil or water samples or perform their own GC analyses which requires highly trained individuals and expensive equipment. Immunochemical assays are reliable methods for the rapid analysis of a variety of compounds (Ngo 1985, Engvall & Pesce 1978), including compounds of environmental concern (VanEmon & Mumma 1990, Vanderlaan et al. 1990, Albro et al. 1979, Blewett & Krieger 1990, Bushway et al. 1988, Fleeker & Cook 1991, Goh et al. 1991, Jung et al. 1989, Thurman et al. 1990). Thus, immunoassays will be able to solve many of the problems of on-site screening, monitoring, and remediating environmental samples.

We have developed a rapid and sensitive immunoassay screening test for one such compound, pentachlorophenol (PCP). PCP is the preservative of choice for treating utility poles and is of environmental concern at many sites. The test kit will detect PCP in soil at 0.5 ppm or above. The assay is field compatible, in that it is simple to perform, requires no specialized training, and can be completed in under 20 minutes including the sample processing step. In addition the test is cost effective, since it allows rapid decisions to be made concerning the treatment of the samples.

MATERIALS AND METHODS

Polyclonal antibodies - New Zealand White rabbits were immunized by intradermal and subcutaneous injections of 500 ug of a PCP analog conjugated to bovine serum albumin in complete Freund's adjuvant. Subcutaneous booster injections of 250 ug of immunogen in incomplete Freund's adjuvant were given at two weeks and then on a monthly basis until a maximum response was obtained (approximately 3 months). Polyclonal antisera were then collected on a routine basis at 7 to 14 days after each boost.

Horseradish peroxidase (HRP) conjugate was prepared with a hydrazide analog of a PCP derivative using the periodate method of Nakane and Kawaoe (1974). HRP (20 mg) was dissolved in 6 mL of 0.1 M $NaHCO_3$ and 2 mL of 0.048 M $NaIO_4$ were added. The reaction mixture was stirred, and after 2 h 1.6 mL of a 1 mg/mL hydrazine analog of PCP in 0.1 M Na_2CO_3 was added. Stirring continued for 2.5 hours. To this mixture was added, with stirring, 0.45 mL of 10 mg $NaBH_4$ in 2 mL of 0.1 M NaOH. The conjugate was dialyzed against phosphate buffered saline (PBS) for 24 h with three changes of buffer. The conjugate was stabilized by lyophilization and

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presented in a unit-dose format by combining the conjugate in the antibody-coated tube.

Antibody coated tubes were prepared by passively coating polystyrene (12X75 mm) NUNC tubes by incubating overnight with rabbit anti-PCP antibody diluted in PBS.

The immunoassay is in the form of a competitive inhibition assay. Basically, in this assay system, the antibody-coated polystyrene tube is used as the solid support. A limiting amount of antibody is attached to the walls of the tube, and PCP in the test sample and the PCP-HRP conjugate compete for these limited binding sites. Therefore, as the concentration of PCP in a sample increases, the amount of PCP-HRP conjugate bound to the tube will decrease. Thus, the color developed by the assay is inversely proportional to the PCP concentration in the sample. Each assay is run in parallel with the standard included in the kit, that is equal to a soil concentration of 0.4 ppm PCP. The color of the test sample is compared to the color of the standard. If the sample contains more color than the standard, then the sample contains less than 0.5-ppm PCP.

The assay procedure involves three steps: Sample processing, immunological procedures, and assay interpretation.

1. Sample processing:
   a. Weigh 10 g of soil, and place it in the extraction vial containing 20 mL of methanol.
   b. Shake vial for 1 min to disperse the soil particles, and extract the PCP.
   c. Filter a small amount of the sample with the supplied filter unit.
2. Immunoassay procedures:
   a. Place 100 uL of sample into the dilution tube containing 1.9 mL of 50 mM KH_2P0_4-K_2HPO_4 (pH 7.4) and 5 mM EDTA (SDB), and mix.
   b. From the dilution tube transfer 100 uL, into the buffer tube containing 0.4 mL of SDB, and mix. The total dilution of the soil sample is 1:200.
   c. Pour contents of buffer tube into antibody coated tube containing the lyophilized enzyme conjugate, and mix.
   d. Incubate 10 min at ambient temperature.
   e. Wash 4X with the wash buffer (PBS with detergent).
   f. Add 200 uL of tetramethylbenzidene (KP Laboratories, Cat. # 50-76-02) from the dropper bottle.
   g. Add 200 uL of hydrogen peroxide (KPL, Cat. # 50-65-02) from the dropper bottle, and mix.
   h. After incubating for 2.5 min stop the color development reaction with 200 uL of 1 M sulfuric acid.
3. Interpretation
   a. Compare the optical density (OD) of the test sample to the OD of the PCP standard that is tested simultaneously with the sample (PCP standard included with the kit). A portable, battery operated photometer (EnSys) was used for the comparison. Visual interpretation is possible but with decreased sensitivity compared to the photometer.
   b. If the sample OD is less than the standard OD, the sample

contains close to or greater than 0.5-ppm PCP

c. If the sample OD is greater than the standard OD, the sample contains less than 0.5-ppm PCP.

d. Other concentration ranges can be assessed. To measure at the 5 and 50 ppm level, the 0.5-ppm diluted sample is serially diluted 10 fold with SDB.

RESULTS AND DISCUSSION

This paper on the assay of PCP in soil describes an immunoassay that we have developed for the environmental field in the competitive, enzyme-linked, immunosorbent assay (ELISA) format. This coated-tube format compares the test samples to the standard that is run in parallel with the sample. This test is field compatible, semi-quantitative, specific, stable, reproducible, and capable of screening for PCP at concentrations as low as 0.5 ppm in soil. These claims are based upon demonstrating the following:

- The sensitivity is 0.5-ppm PCP in soil
- High recovery is obtained from spiked samples (consistent sensitivity)
- Different soil matrixes cause little interference in the assay
- The antibody is highly specific (little cross-reactivity to other compounds)
- The test is reproducible from lot-to-lot, day-to-day and person-to-person
- The assay is compatible with a temperature range of 15-30° C
- Storage at room temperature (up to 30° C) is acceptable
- Good performance was demonstrated with field samples.

The development of a soil test that can detect 0.5-ppm PCP is sufficiently sensitive to meet the limits imposed by regulatory agencies. To develop a test at this level, the concentration of the standard was adjusted such that samples containing 0.5 ppm or more PCP were positive. In addition, it was desirable for the standard to be in the middle of the dose response curve since that portion of the curve is the most precise. Figure 1 shows that the midpoint of the dose response curve is 0.4 ppm which was the value determined for the kit standard. Figure 2 shows that 0.5 ppm PCP spiked into buffer produces a positive test greater than 95 % of the time which demonstrates that the sensitivity of the test is 0.5 ppm.

The sensitivity, as determined by the concentration at which almost all of the samples produce a positive test, will be shifted if soil matrix interference exists in an assay. In order to determine the impact of matrices on the sensitivity of this test, eleven different negative soil extracts were spiked gravimetrically with PCP. Figure 2 demonstrates that greater than 95 % of the samples are positive at the 0.5 ppm and higher levels of PCP. As the PCP concentration decreases the probability of a negative result increases. Practically all samples at or below 0.125 ppm PCP are negative. Therefore, the sensitivity of this assay system will detect samples with PCP at 0.5 ppm or higher and will be negative if the concentration of PCP is below 0.125 ppm.

Additional data demonstrating the absence of matrix effect was collected by testing negative soil samples obtained from different parts of the United States. The eleven different soil samples tested negative. In fact, the signals from the assays with negative soils were not significantly different compared to negative buffer. It is concluded that soil did not interfere with the assay.

Another criterion in demonstrating that the PCP immunoassay is suitable for

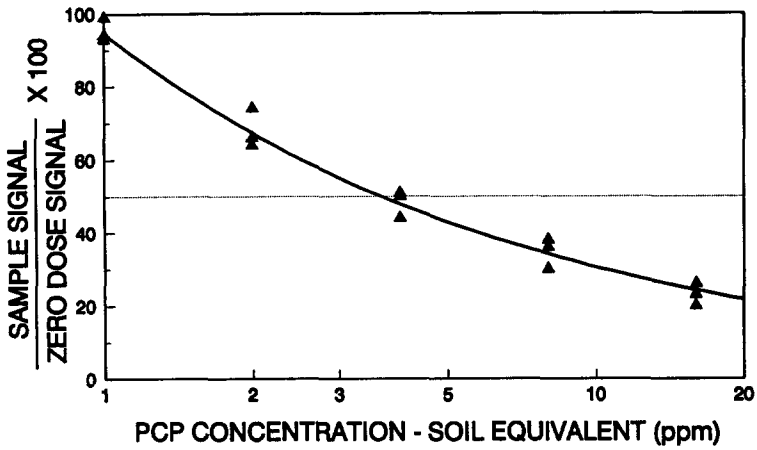

Figure. 1. PCP concentration vs. colorimetric response
The immunoassay was performed according to the protocol. In addition to the concentration shown in the figure, a zero PCP dose was tested. The signal at each of the doses was divided by the signal of the zero PCP dose. This value was then plotted against the concentration of PCP.

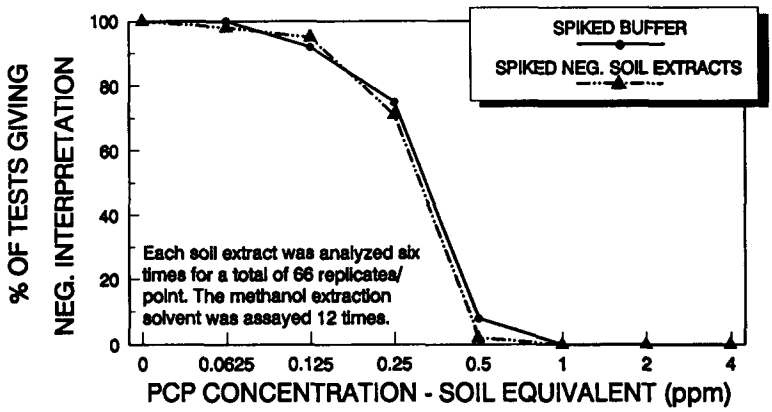

Figure. 2. Matrix effect of negative soil samples
Eleven negative soils were extracted and spiked with the indicated concentrations of PCP. The extraction solvent was also spiked. The spiked materials were treated as samples and assayed.

field testing environmental samples was to determine the degree of interference from compounds that might be found on sites contaminated with PCP. Cross-reactivity was determined by testing all of the compounds at 1000 ppm dissolved in methanol. Compounds that demonstrated cross-reactivity were tested further to determine the minimum concentration that resulted in a positive test compared to the kit standard. Tetrachlorophenol was the only compound tested in Table 1 that showed significant cross-reactivity. This demonstrates that the antibody is highly specific for PCP and little interference would be anticipated from synthetic organic chemicals that might be found at the same site as PCP.

Along the lines of cross-reactivities, it was important to investigate diesel fuel in more detail since diesel with 6% PCP is usually the method for treating

wood products. This assay was not affected by soil contaminated with 10 % diesel fuel. The recovery of PCP from soil contaminated with 10 % diesel and 5 ppm PCP was virtually 100 %; it was concluded that the immunoassay functions well in the presence of diesel.

Table 1. Cross-reactivity of PENTA RISc™ soil immunoassay.

	Concentration in soil to cause positive test result
PCP	0.5 ppm
Phenol	>1000 ppm
4-Chlorophenol	>1000 ppm
2,4-Dichlorophenol	>1000 ppm
2,6-Dichlorophenol	700 ppm
2,3,4-Trichlorophenol	400 ppm
2,4,5-Trichlorophenol	100 ppm
2,4,6-Trichlorophenol	16 ppm
2,3,5,6-Tetrachlorophenol	1.2 ppm
Tetrachlorohydroquinone	500 ppm
Pentachlorobenzene	>1000 ppm
PCB (Aroclor 1254)	>1000 ppm
CCA (copper, chromium arsenate)	>1000 ppm
Creosote	>1000 ppm
Diesel fuel	No interference at 10 % w/w (diesel/soil)

Reproducibility from person-to-person, day-to-day, and lot-to-lot were investigated. Little difference between the results of two people performing the assay was observed. A comparison between three lots of product demonstrated excellent lot-to-lot reproducibility. The assays for the different lots were performed on different days. Therefore, if day-to-day variation occurred, it would be shown with this set of data; but it was not seen. These experiments indicate that the product is reproducible and not subject to inter-person, day or lot variations.

Acceptable assay temperatures were determined to be between 13-30° C. When tested at 4° C the test became more sensitive and less precise. At 37° C the sensitivity was approximately the same as assays performed at lower temperatures, however, intra-assay precision of interpretation deteriorated in comparison to the precision observed at lower temperatures; therefore the test is not recommended to be performed at or above 37° C.

The test has shown little loss in performance when the assay materials were stored near room temperature (about 20° C) or at 37° C for 2 months. Regression analysis evaluating signal decay and performance suggest long-term stability (>6 months) when stored at room temperature (Figure 3).

The ultimate test of any assay development process is to correlate field sample results using the new assay to the accepted standard testing methods, in this case the immunoassay versus GC or GC-MS. Table 2 shows the comparison between our immunoassay screening test and GC-MS and GC

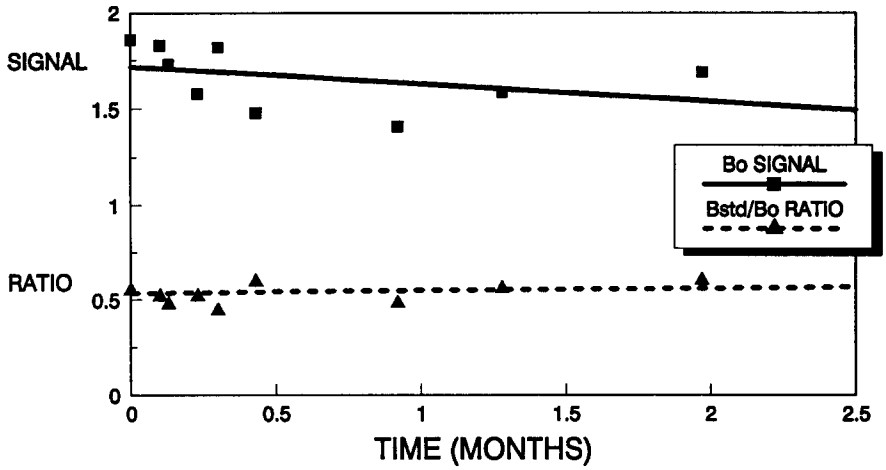

Figure. 3. Penta RISc™ soil test kit stability

The stability of the test kit was determined at the time points indicated stored at room temperature (20-25° C). The Bo signal is the signal at zero dose. The ratio of Bstd/Bo is calculated and monitors changes in the immunochemistry.

results. The results show excellent correlation with the possible exception of two samples. Sample 312-6 was a false positive when the immunoassay was compared to the GC methods. However, the detection limits for the GC-MS methods are 1 ppm or higher. While this may indicate a false positive result, it could also indicate that the sample contained PCP in the range of 0.5-1 ppm PCP. Sample 312-5 was slightly less than 50 ppm according to the GC methods, but the immunoassay suggested that it was close to or greater than 50 ppm. If the value from the GC is precisely correct then this sample is a false positive, and as explained earlier, the interpretation is biased toward false positive results. Therefore, it would not be surprising to detect a sample at 46 ppm. Even considering these potential outliers the immunoassay and the reference analytical methods demonstrated excellent correlation. Correlation to this extent justifies the use of this test for screening field samples for PCP.

The test kit has many advantages, the most important are its field compatibility, speed in obtaining a result, and lack of false negative results. To have these advantages certain compromises had to be made. The first compromise was to bias the test to minimize false negative results. A false negative test result does not detect contamination in a sample and therefore, is potentially dangerous to the customer and the environment. A false positive, on the other hand, might be inconvenient but not dangerous. Therefore, the test kit has been designed to detect almost all positive samples containing greater than 0.5-ppm PCP with a small number of false positive results.

The other compromise was to simplify the immunoassay by making it semi-quantitative. This was done by eliminating all but one standard, and limiting the ability of the test to quantitate samples. Using the one standard greatly simplifies the test protocol and eliminates all of the data manipulation and expensive instrumentation that are normally necessary for quantitative immunoassays. The test becomes a semi-quantitative, yes/no, test that

determines whether a sample is below 0.5 ppm, between 0.5 and 5 ppm, between 5 and 50 ppm, or greater than 50 ppm PCP in soil. This ranging of the concentration of a sample is accomplished by dilution of the sample using a common immunochemistry with detection always occurring at the most precise point of the dose response curve.

Table 2. Comparison between the immunoassay and reference GC/MS methods.

	Reference Concentration (ppm)		
	GC-MS	GC	
Sample No.	EPA3540/8270	EPA 8040	PENTA RISc™
312-1	1.7	1.3	0.5-5
312-2	7.2	5.7	5-50
312-3	12	6.1	5-50
312-4	2.5	1.3	0.5-5
312-5	46	-	>50
312-6	nd*	nd**	0.5-5
312-7	22	21	5-50
321-1	3.2	3.3	0.5-5
321-2	5.3	2.5	5-50
321-3	11	-	5-50
405-1	20	17	5-50
405-2	33	33	5-50
405-3	56	52	>50
405-4	65	65	>50
405-5	74	74	>50

* Practical limit of detection was 1.8 ppm
** Practical limit of detection was 1 ppm

While the compromises impose certain limits on the test, they make possible the rapid screening of samples in the field. The rapid screening of multiple samples is a significant advantage compared to the standard analytical GC or GC/MS methods. The almost instant screening of a sample allows important questions to be answered concerning the project, e.g. has the plume of contaminated soil been located, has the area been remediated to acceptable levels, does additional soil need to be removed, etc.?

This paper has shown that we have developed and validated a rapid, easy-to-perform, sensitive, and stable immunoassay that is capable of detecting PCP in soil at 0.5 ppm or higher concentrations. Because the test is simple to conduct and requires less than 20 minute to perform, it is applicable to field testing and will aid in on-site mapping, monitoring, and remediation. In addition, the ability to rapidly test, on-site, makes this test cost-effective by allowing the more efficient use of people, time and equipment resources.

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