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EVALUATION OF IMMUNOASSAY-BASED FIELD TEST KITS FOR THE DETECTION OF PETROLEUM FUEL HYDROCARBONS IN SOIL*

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ABSTRACT

The objectives of this project are to identify, experimentally evaluate and implement the use of alternative field screening methods that are specific for environmental contaminants of interest and concern to the Department of Energy. Immunochemical techniques are rapidly becoming a significant component in the arsenal of field screening methods. Analytical results obtained by immunoassay have been shown to correlate well with those obtained by traditional laboratory methods. Also, the use of immunoassay-based field screening methods can significantly reduce the cost and time required for environmental assessment. We are currently evaluating the effectiveness of several immunoassay-based test kits for detecting petroleum fuel hydrocarbons in soil. Evaluations of two kits, one a semiquantitative assay and the other a quantitative assay, have been completed. The samples analyzed were either solvent or soil spiked with either a mixture of benzene, toluene, ethylbenzene and the three isomers of xylene (BTEX), or gasoline. The kits performed well and according to the manufacturers' claims. Of the 50 assays made with the semiquantitative test, the concentrations of 44 samples were correctly determined. The other six samples were determined to be false positives. A soil matrix effect was observed that could account for some of the false positive results. Experimental results using the quantitative test with BTEX (68 assays) correlated well with those expected; R^2 of 0.976 to 0.983 with slopes of 0.94 to 0.97. With gasoline (38 assays) R^2 values of 0.957 to 0.987 and slopes of 0.76 to 0.78 were obtained. The lower slopes with gasoline are indicative of the lower immunoreactivity of that particular sample of gasoline relative to BTEX.

INTRODUCTION

Sample analysis is a major component of environmental restoration and waste management activities. Standard laboratory methods are both expensive and time-consuming. A major portion of the field samples taken to the laboratory for analysis either are negative for the analyte being tested, or are contaminated below the regulated level. Effective field screening methods could eliminate much of this effort and expense.

An objective of this program is to define commercial screening methods that are capable of measuring environmental contaminants of concern to the Department of Energy.[1,2] The approach involves selecting potentially useful technologies, experimentally evaluating the methods that utilize the technology, and transferring the validated methodology to appropriate users. One aspect of the latter involves submission of the protocols and performance data for inclusion in the DOE Methods for Evaluating Environmental and Waste Management Samples. Fribush and Fisk[3] recently reviewed the role of field analytical methods in the Environmental Protection Agency's Superfund project.

Accounts of our experiences with immunoassay (IA)-based test kits for polychlorinated biphenyls (PCBs) and mercury have previously been presented[4-7]. This paper describes our experiences with two different IA-based test kits for petroleum fuel hydrocarbons in soil.

EXPERIMENTAL METHODS

Sample preparation

For these studies, both spiked solvent, and spiked soil, samples were analyzed. Samples were spiked with either a standard BTEX mixture (equal parts of benzene, toluene, ethylbenzene and the 3 isomers of xylene) obtained from AccuStandard, Inc., or a gasoline standard, API-91-01 from the American Petroleum Institute. A standard soil, obtained from the Rocky Mountain Arsenal and designated RMA[8], was used in most experiments. Soil extractions and solvent spikes were made in the solvent recommended by the manufacturers of the specific kit being used, usually methanol. Soils were extracted according to the instructions with the kits. In most cases this involved shaking the soil with an equal volume of solvent for 1 to 2 min, followed by filtration. When required, dilution of the soil extracts and spiked solvents for assay was done according to the kit instructions.

Immunoassays

The general procedure for an immunoassay is as follows. Aliquots or dilutions of the samples are buffered and added to the immobilized antibody, together with a constant amount of the conjugate (a BTEX component/enzyme complex). The antibody must be immobilized so that unbound analyte and conjugate can be removed by rinsing. The antibody is immobilized in different ways by different manufacturers. Competition between analyte and conjugate for binding to the antibody is allowed to occur. The more analyte present the less conjugate will bind. The amount of bound conjugate is determined by the addition of appropriate substrates which are enzymatically converted to colored products. The amount of color produced is proportional to the amount of conjugate that was bound to the antibody. The assay is a typical competitive immunoassay wherein the amount of color in the final reaction is *inversely* proportional to the amount of BTEX-related components in the test sample.

The kits evaluated in this study were obtained from Millipore Corporation and Ohmicron Environmental Diagnostics. Both kits utilize a similar format in that the analyte(antigen) and conjugate are exposed in solution to the antibody, the color reaction is performed in solution and read by spectrometry.

The Millipore kit has the antibodies immobilized onto the surface of test tubes. Conjugate and analyte are added to the tubes and allowed to compete for binding to the antibody. Excess analyte and conjugate are rinsed away, enzyme substrates are added, and after an appropriate time the color is read in a spectrophotometer. Standard solutions of BTEX are used in each assay, e.g., 0, 2, 10 and 50 ppm. With reference to these standards, a semiquantitative estimate of the BTEX content of the test samples can be made. For example, a soil sample with an absorbance greater than the 2 µg/mL standard would be estimated to contain less than 2 µg/g BTEX equivalents; samples with absorbance less than the 2 µg/mL standard but greater than the 10 µg/mL standard would be estimated to contain 2 to 10 µg/g BTEX equivalents; and so forth. Accordingly, test sample results can be interpreted as being less than 2, 2 to 10, 10 to 50, or greater than 50 µg of BTEX equivalents per gram of soil.

The Ohmicron test kit is similar to the Millipore kit with the following exceptions: 1) The antibodies are attached to magnetic beads and are retained in the tubes during rinsing by the use of a magnetized tray. 2) The color produced in the tubes is read in a programmable spectrophotometer; based on the absorbance of standard samples included in the assay, a

concentration curve is established from which the concentration of the test sample can be read directly off the instrument. In this way, quantitative results are obtained with this test kit.

RESULTS AND DISCUSSION

Soil extraction

Materials to extract soil samples are available from both Millipore and Ohmicron as separate kits. The Millipore kit measures the soil sample to be extracted by direct weighing. The Ohmicron kit uses a syringe-like collection device, collecting a volume of soil which can be related back to weight. With either method the soil is quickly transferred to an extraction vessel containing the extraction solvent. Each method has drawbacks, e.g., collecting by weight would be more subject to losses of the volatile analytes, while collecting by volume would be more variable unless the extraction vessels are tared and reweighed after introducing the soil. [Because spiked and not field soil samples were used in these studies, all soil samples were measured by weight.] Five (Millipore) or 10 (Ohmicron) gram soil samples were used, extracting with equal volumes of extractant. The Millipore extraction vessels contain stainless steel dispersion balls. These are useful for sticky clay soils which are otherwise difficult to disperse. Each kit utilizes some type of filtration unit to clarify the extracts. A practical drawback to this is the fact that none of the filtration methods produces a quantitative solid/liquid separation, leaving the user with a mixture of contaminated solvent and soil for disposal.

Assay performance

Millipore. A total of 22 independent samples of BTEX-spiked methanol were assayed. Concentrations ranged from 0 to 120 ppm. Results were interpreted relative to BTEX standards of 0, 2, 12 and 60 µg/mL. Nineteen of the samples were correctly interpreted and 3 were determined to be false positives.

A duplicate set of eight soil samples were spiked with BTEX at concentrations ranging from 0 to 100 ppm. A single assay was done on each of the 16 samples. One of the 50 ppm samples was incorrectly interpreted as being > 60 ppm, a false positive. All the others were correctly interpreted, indicating good reproducibility of the assay of duplicate samples.

Six soil samples were spiked to gasoline levels in the range of 1 to 80 ppm. Extracts of these soils were independently assayed twice and the results were interpreted as gasoline. [Millipore has empirically determined that 2, 10 and 50 ppm BTEX is equivalent to 2, 11, and 58 ppm gasoline.] Two of the 12 analyses were found to be false positives, i.e., both analyses of the 10 ppm sample were interpreted to be 11 to 58 ppm. The other 10 analyses were correctly interpreted.

Overall, the data showed good accuracy and reproducibility of the method. A total of 44 individual samples were tested, 22 spiked methanol solutions and 22 spiked soils. Six of the individual spiked soils were analyzed in duplicate for a total of 50 analyses. Of the 50 analyses, six (12%) were incorrectly interpreted as being false positives; no false negatives were observed. Most of the false positives were at concentrations equivalent to a standard. In this situation, it is unlikely that the absorbance of the test sample would exactly match that of the calibrator and thus false positive, or false negative, interpretations would be statistically more prevalent than correct interpretations.

A soil matrix effect on the Millipore test was observed that could contribute to the number of samples interpreted as false positives. This was shown by using three different types of soil to determine the recovery of spiked gasoline. Duplicate 5 g samples of each soil type were spiked with methanol (0 ppm gasoline/g) or with gasoline in methanol (10 ppm gasoline/g) and extracted with 5 mL of methanol. One soil sample, a "clay", required the use of 10 mL of methanol; test results were corrected for this dilution. Each extract was tested in duplicate along

with the BTEX calibrators equivalent to 0, 2, 11 and 58 ppm gasoline. The results show that each of the extracts of blank soils produced less absorbance than the 0 ppm standard. [In order to assess the effects of soil matrices on gasoline recovery, it was necessary to make quantitative determinations of the amount of gasoline recovered. These quantitative determinations were obtained from a standard curve generated from the absorbance values of the test standards. The linear calibration curve was derived by a least-squares fit of the ratio $(B/B_0) \times 100$ (%) versus the log of the concentrations of the standards, where B_0 = the absorbance value (A_{450}) of the 0 ppm standard and B = the absorbance values (A_{450}s) of the positive standards. Estimates of the spiked soil concentrations were made using the B_0 values of both the 0 ppm standard and the blank soil samples.] Based on percent of the 0 ppm standard, there was the equivalent of 0.9 to 1.4 ppm of gasoline in the blank soils. On the same basis, the gasoline content of the three types of spiked soils ranged from 11.6 to 21.9 ppm. Clearly, the level of gasoline detected in the blank soils does not account for the excess recovered in the spiked soils. (NOTE: Other laboratory analyses of these soils did not indicate the presence of fuel hydrocarbons.) If B_o values based on the A_{450} values for the blank soils are used to estimate the gasoline content of the spiked soils, the results are in the expected range of 7.5 to 13.5 ppm. Although it is impractical to run blank soil samples in the field, these data indicate that by using B_o values based on the 0 standard provided in the kit, the gasoline content of the soil is likely to be overestimated. With the semiquantitative use of the test, this would only be a problem when the fuel hydrocarbon content of the test soil is near that of a calibrator. In those cases, false positive interpretations could prevail.

Ohmicron. A total of 19 independent samples of BTEX-spiked extraction solution were prepared (0 to 250 ppm) and 14 of these were analyzed in duplicate - a total of 33 analyses. The concentrations of BTEX found by the immunoassay (IA) were plotted versus the expected concentrations. Statistically, the IA results gave a best-fit straight line with an $R^2 = 0.983$ and a slope of 0.97, showing good agreement with the concentrations expected.

Twenty five independent BTEX-spiked soil samples (0 to 200 ppm) were analyzed, 10 of them in duplicate. Results of the 35 analyses gave an $R^2 = 0.976$ and a slope of 0.94, similar to the values obtained for spiked extraction solution.

Four gasoline-spiked extraction solution samples (1 to 150 ppm) were analyzed in duplicate. The best-fit straight line had an $R^2 = 0.987$ and a slope of 0.78. The slope is indicative of the lower immunoreactivity of gasoline relative to that of BTEX.

Several assays using gasoline-spiked soil were done. In one experiment soil samples were spiked in duplicate at ten concentrations (2.5 - 200 ppm) and each extract assayed once. In another experiment, five soil samples were spiked (12.5 - 150) and the extracts assayed in duplicate. A composite of the results of all 30 analyses gave an R^2 and slope of 0.957 and 0.76, respectively. Again the lower immunoreactivity of the gasoline used, relative to BTEX, was observed.

Overall, the results show that the test accurately and reproducibly measured BTEX in extraction solution and soil with R^2 values of 0.976 to 0.983 and slopes of 0.94 to 0.97. They also show that gasoline is reproducibly measured in extraction solution and soil with R^2 values of 0.957 to 0.987. Slope values of 0.76 to 0.78 indicate that the gasoline standard used, API-91-01, is measured about 75% as efficiently as BTEX, undoubtedly due to differences in immunoreactivity. After adjusting for this difference, however, gasoline is also accurately measured in this test.

Although a detailed evaluation of potential matrix effects on the Ohmicron test was not made, extracts of blank soils consistently produced less color than the 0 ppm standard. This could lead to an overestimation of the petroleum fuel hydrocarbon content in soil samples that contain low levels of analyte.

The sensitivity of either kit for BTEX in solvent is in the range of 1 to 2 ppm. In soil, this is confounded somewhat by a matrix effect that indicates the presence of small amounts of immunoreactive substance in BTEX negative soils. Consequently, blank soils having been spiked

with 1 to 2 ppm BTEX will test as having a slightly higher concentration. The sensitivity for gasoline is moderately less than for BTEX.

Other kits. Both the Millipore and Ohmicron kits are based on a similar format in which the color reaction is developed in solution and read by spectrometry. Other kits are available that utilize a different format in which the color reaction is developed on a solid surface (membrane) and is read by reflectometry. Our experience to date indicates that the latter format is significantly less accurate and reproducible than the former.

CONCLUSIONS

Both of the test kits used in this evaluation were found to provide rapid, easy to use, accurate and reproducible assays for petroleum fuel hydrocarbons in soil. The use of such field screening kits could decrease the time and cost of assessment and remediation of contaminated sites.

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KEY WORDS

Immunoassay, Petroleum fuel hydrocarbons, BTEX, Soil