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Project Summary

Superfund Innovative Technology Evaluation (SITE) Report for the Westinghouse Bio-Analytic Systems Pentachlorophenol Immunoassays

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The results of the demonstration of two Westinghouse Bio-Analytic Systems (WBAS) immunoassay technologies are described in this report. The immunoassays measure parts per billion concentrations of pentachlorophenol in environmental water samples. The study was conducted under the Superfund Innovative Technology Evaluation (SITE) Program and designed to evaluate the ruggedness and utility of a semiquantitative immunoassay field kit. Results obtained from the field kit were compared to those obtained from a quantitative, high-sample-capacity plate immunoassay. Both techniques were compared to a standard U.S. Environmental Protection Agency (EPA) gas chromatography/mass spectrometry (GC/MS) procedure (EPA Method 8270) for pentachlorophenol determination.

The results of the WBAS immunoassay demonstration support the conclusion that the field immunoassay is a useful screening tool. The demonstration verified that the method can provide qualitative or semiquantitative screening information. Although the results were more variable than had been anticipated, the incorporation of additional procedural precautions and carefully chosen quality control acceptance criteria for on-site analysis could improve performance substantially. Both immunoassays produced results biased high compared to the GC/MS results, but the tendency was not large and may have been partly due to loss during sample extraction (EPA Method 3510) prior to analysis by GC/MS. The detection of structurally related compounds by the immunoassays may have also contributed to the high bias. The results indicate that the plate immunoassay is an accurate and precise method for quantitating pentachlorophenol in water.

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

This evaluation report presents the results of a demonstration designed to assess the capabilities of two immunoassay technologies to measure pentachlorophenol (PCP) in water. The technologies, a semiquantitative field kit immunoassay and a quantitative plate immunoassay, were both developed by Westinghouse Bio-Analytic Systems (WBAS)* of Rockville, Maryland. The demonstration was conducted under the Monitoring and Measurement Technologies Program as part of the U.S. Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation (SITE) Program. The demonstration was conducted under the guidance of the EPA Environmental Monitoring Systems Laboratory in Las Vegas, Nevada (EMSL-LV).

Description of Technologies

Immunoassays are analytical techniques based on protein molecules (antibodies).

* Mention of trade names or commercial products does not constitute an endorsement or reccomendation for use.

The binding of a specific antibody to its target analyte can be used to quantitatively or qualitatively determine the extent of contamination in environmental samples. Specific antibodies have been developed to detect single analytes or groups of related compounds. The WBAS kit immunoassay, based on rabbit polyclonal antisera adsorbed on 8-well, polystyrene, microtiter strips, has a reported detection limit for PCP of 3 parts per billion (ppb), a linear dynamic range of 3 to 40 ppb, and a total analysis time of 30 minutes per sample. It requires minimal logistical requirements for on-site analyses. The WBAS 96-well plate immunoassay, based on a rat monoclonal antibody, has a reported detection limit of 30 ppb and a linear dynamic range of 50 to 400 ppb. It requires 3 hours of hands-on analysis time per plate (10 to 20 samples run in triplicate) and involves certain logistical considerations (e.g., a mobile laboratory) for on-site analyses. A previous evaluation by the EMSL-LV compared the plate immunoassay to GC results for PCP analysis; data from this SITE immunoassay demonstration complement the previous study.

The WBAS kit immunoassay was demonstrated under field (on-site) and laboratory (off-site) conditions to determine its ruggedness, reliability, and potential for use as a rapid, on-site, analytical tool in the Superfund Program. The results obtained from the kit immunoassay analyses performed on-site and off-site were compared to those generated off-site by the plate immunoassay; both immunoassay techniques were compared to standard EPA gas chromatography/mass spectrometry (GC/MS) methods for the analysis of PCP (EPA Method 3510 sample extraction followed by EPA Method 8270 analysis by GC/MS).

PCP Immunoassay Demonstration Design

The on-site demonstration took place in July and August, 1989, at the MacGillis & Gibbs Superfund Site in New Brighton, Minnesota, a National Priorities List site known to have ground water contaminated with PCP. The immunoassay demonstration was coordinated through RREL and conducted jointly with a SITE demonstration of a bioremediation technology designed by BioTrol, Inc. (Chaska, Minnesota), to biodegrade PCP in aqueous waste streams. The design of the immunoassay SITE demonstration involved several planning components: predemonstration tests, an experimental design, a sampling and analysis design, quality assurance and quality control (QA/QC) planning, and data base management.

Field samples for the immunoassay demonstration were obtained from three sampling points in the bioreactor system: influent samples collected before pretreatment (nutrient addition and pH adjustment), influent samples collected after pretreatment, and effluent samples collected before filtration. Samples were collected over three 1-week periods which coincided with a 1-, 3-, and 5-gallon-per-minute flow rate of the ground water through the bioreactor. Composite and grab samples were collected, homogenized, split, and analyzed on-site with the kit immunoassay. Sample splits were analyzed at the WBAS and EMSL-LV laboratories with the kit and plate immunoassays and with GC/MS by Science Applications International Corporation in San Diego, California. Comparison of the analysis results by each method and analysis site was a critical component in the evaluation of the immunoassays.

A rigorous QA project plan was implemented at all sites involved in the study. This plan included the analysis of a battery of QA/QC samples, including duplicate, split, matrix spike, QA audit, QC performance, field blank, negative control (NC) samples and cross-calibration standards. The QA/QC samples were used to assess the performance characteristics of the two immunoassay methods and to test the capabilities of the technologies to meet the stated data quality objectives (DQOs) of the demonstration; the most critical DQO was that the immunoassay sample results had to be within a factor of two (50 to 200%) of the GC/MS results. Traditional methods such as GC/MS have interlaboratory biases of 30 % or more in addition to other sources of variability. Thus, the use of a factor of two for the immunoassay implies a slightly greater (but quite useable) variability than one might expect from the more traditional methods. All bioreactor and QA/QC sample data from all analysis locations were subjected to EMSL-LV QA review and verification. The data were then entered and stored in a documented data base.

Method Results and Comparisons

The immunoassay technologies were assessed by comparing the analyses of the bioreactor influent and effluent samples. Because of the differences in sample ranges of the influent and the effluent samples, results from these sample types were treated separately in data evaluation. The most critical method and analysis site comparisons were: (1) the on-site kit immunoassay to the GC/MS, (2) the on-site kit immunoassay to the plate immunoassay, and (3) the plate immunoassay to the GC/MS.

The on-site kit immunoassay performed well as a semiquantitive screening method. When compared to the results from the GC/MS analysis, there was good relative (rank order) agreement between the two methods. Fourteen of the 16 influent samples analyzed on-site were within the factor-of-two DQO over a concentration range of approximately 1 to 60 ppm PCP. The effluent samples analyzed by the two methods were in the same general concentration range (kit immunoassay = 0.2 to 2.3 ppm; GC/MS = 0.008 to 0.9 ppm). Results of influent and effluent samples indicated a consistent tendency for the kit immunoassay data to have a high bias when compared to the GC/MS data. This bias may be due to extraction inefficiency of EPA Method 3510, cross-reactivity of tetrachlorophenol in the immunoassay, or a combination of these and other factors. Kit immunoassay results for influent samples averaged from 65 to 119 % higher than GC/MS results, depending on analysis site. Effluent sample bias was small in practical (ppm) terms. The positive bias suggests that the kit immunoassay has a minimal tendency to generate false negative responses.

The kit immunoassay results were compared to the plate immunoassay results to detect differences between the methods and to provide insight for interpreting the performances of the immunoassays compared to the GC/MS. There was reasonable agreement between the two immunoassay techniques; 27 of 38 (71 %) on-site kit immunoassay influent sample results were within a factor of two of the plate immunoassay results (WBAS and EMSL-LV analysis sites combined). For both immunoassays, effluent samples were in the same general range (0.20 to 2.74 ppm; n = 38). Although no significant bias was observed between the two immunoassay techniques, a significant amount of scatter (variability) was observed.

Overall, the plate immunoassay results compared more favorably to the GC/MS than did the kit immunoassay results. At one analysis site (EMSL-LV), 17 of 18 (94 %) effluent sample results were within a factor of two of the corresponding GC/MS results, while at the other site (WBAS), 12 of 18 samples were within this limit. The results from various QA/QC samples suggest that WBAS had unusual site- or operator-specific factors affecting the quality of their analyses. As with the kit immuno-

assay, the plate immunoassay exhibited a high bias when compared to the GC/MS, although the bias was much smaller (17 to 40 % for influent samples, depending on analysis site).

Quality Assurance and Quality Control Results

Data derived from the QA and QC samples provided insight into the intra- and intermethod performance assessment in terms of the accuracy and precision of the kit and plate immunoassays. Seventy-six percent of the audit samples and 74 percent of the bioreactor samples analyzed using the kit immunoassay met the accuracy DQOs. The false negative rate was 2.6 percent (based on 76 effluent and influent sample analyses), and the false positive rate was 19 percent (based on 98 NC samples). However, the matrix spike recoveries were unsatisfactory (-166 to +313 %), a fact that may be attributed to a poorly developed matrix spike protocol. Precision for the kit immunoassay was not as good as expected. The coefficients of variability for QC performance and QA audit samples exceeded the DQO of ±50 % in most cases; however, results of the duplicate and split sample analyses were reasonably good for a semiquantitative method.

Ninety-five percent of the audit samples and 81 percent of the bioreactor samples met the accuracy DQO for the plate immunoassay. There were no false negatives (based on 78 effluent and influent sample analyses) and no false positives (based on 21 NC samples). The matrix spike recoveries were less than satisfactory (41 to 169 %), but were considerably better than for the kit immunoassay spike recovery results. Overall, precision for the plate immunoassay method was better than the kit immunoassay method. Better precision and accuracy for the plate immunoassay was not surprising because the kit immunoassay was designed to be a semiquantitative method while the plate immunoassay was expected to be quantitative.

Conclusions and Recommendations

The WBAS kit immunoassay proved to be a useful and promising technology that can provide on-site, real-time, cost-effective, semiquantitative data with a low risk of generating false negative responses. The kit immunoassay, which is easy to learn and perform in the field, can be an effective field screening method at Superfund sites known to have PCP-contaminated water. The plate immunoassay exhibited better precision and accuracy than the kit immunoassay, with quantitative results closer to those generated by the GC/MS. The plate immunoassay can be readily set up in a field laboratory, and its sample output is greater than that of the kit immunoassay. The SITE demonstration indicated that the WBAS kit and plate immunoassay technologies can provide effective screening capabilities in the field and can be used to complement conventional laboratory methods for measuring PCP in aqueous samples. The demonstration also underscored the need for continued QA/QC guidelines and protocol development to improve and fully characterize the quality of immunoassay data. Both WBAS immunoassays evaluated in this report showed promise as measurement and monitoring tools at hazardous waste sites.

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The complete report, entitled "Superfund Innovative Technology Evaluation (SITE) Report for the Westinghouse Bio-Analytic Systems Pentachlorophenol Immunoassays," (Order No. PB92- 188 713/AS; Cost: $26.00; subject to change) will be available only from:

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