



Innovative Technology Verification Report

PCP Immunoassay Technologies



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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Executive Summary

This innovative technology evaluation report (ITER) presents information on the demonstration and evaluation of three immunoassay field screening technologies for determining pentachlorophenol (PCP) contamination in soil and water. These technologies were demonstrated in Morrisville, North Carolina, in August 1993. The demonstration was conducted by PRC Environmental Management, Inc. (PRC), under contract to the Environmental Protection Agency's (EPA) Environmental Monitoring Systems Laboratory-Las Vegas (EMSL-LV). The demonstration was developed under the Monitoring and Measurements Technologies Program (MMTP) of the Superfund Innovative Technology Evaluation (SITE) Program.

The three immunoassay technologies evaluated during this demonstration were (1) the Penta RISC Test System developed by EnSys, Inc., (2) the Penta RaPID Assay developed by Ohmicron Corporation, and (3) the EnviroGard Pentachlorophenol (PCP) Test Kit developed by Millipore Corporation. These technologies were demonstrated in conjunction with the demonstration of two other field screening technologies: the HNU-Hanby Test Kit developed by HNU Systems and the Field Analytical Screening Program (FASP) PCP Method developed by EPA's Region 7 under the Superfund Program. The demonstrations of these other two technologies are presented in separate reports similar to this one.

The first objective of this demonstration was to evaluate each of the field screening technologies for accuracy and precision in detecting high and low levels of PCP in soil and water samples by comparing their results to those attained by a confirmatory laboratory using standard EPA analytical methods. These EPA-approved methods are used to provide legally defensible analytical data for the purpose of monitoring or for the enforcement of environmental regulations. Because these EPA-approved methods are used by the regulatory community, this demonstration also used these methods. Though these methods may include inherent tendencies which may bias data or may include procedures with which developers disagree, they are the best methods for providing legally defensible data as defined by the regulatory community. To remove as much of these inherent tendencies as possible, PRC used post-hoc residual analysis to remove data outliers. Each technology also was qualitatively evaluated for the length of time required for analysis, ease of use, portability, and operating cost.

The second objective of the demonstration was to evaluate the specificity of each technology. The evaluation of specificity was performed by examining any problems due to naturally occurring matrix effects, site-specific matrix effects, and chemical cross reactivity. Information on specificity was gathered from each developer, from the analysis of demonstration samples, and from a specificity study performed during the demonstration.

The site selected for demonstrating the technologies was the former Koppers Company (Koppers) site in Morrisville, North Carolina. One of the reasons this site was selected was because a National Risk Management Research Laboratory (NRMRL) SITE demonstration was occurring simultaneously, thus allowing EMSL-LV and NRMRL to combine logistical and support efforts. Another reason for selecting the former Koppers site was that historical documentation indicated its PCP contamination ranged from none detected to 3,200 parts per million (ppm) in soil and from none detected to 1,490 parts per billion (ppb) in groundwater. The PCP carrier used at this site was a mixture of isopropyl ether and butane. Soil and water samples also were collected from the Winona Post site in Winona, Missouri. These samples were shipped to the former Koppers site for inclusion as demonstration samples. Winona Post samples were included to broaden the scope of the demonstration

by introducing a different sample matrix and PCP carrier, diesel fuel, to the evaluation of each technology. Findings for each of the immunoassay field screening technologies demonstrated are summarized below.

EnSys, Inc.: Penta RISc Test System

The Penta RISc Test System is designed to quickly provide semiquantitative results for PCP concentrations in soil and water samples. In its standard format for soil analysis, the semiquantitative ranges assessed are: greater than 50 ppm, between 50 and 5 ppm, between 5 and 0.5 ppm, and less than 0.5 ppm. This technology's ranges for water analysis are greater than 5,000 ppb, between 5,000 and 500 ppb, between 500 and 5 ppb, and less than 5 ppb. The developer will customize these ranges to a user's needs. The immunoassay chemistry produces compound-specific reactions to PCP allowing its detection and quantitation. Polyclonal antibodies are fixed to the inside wall of a test tube where they offer binding sites for PCP. An enzyme conjugate containing a PCP derivative is added to the test tube to compete with PCP from samples for antibody binding sites. Excess sample and enzyme conjugate are removed from the test tube by washing, and reagents are added to the test tube to react with the enzyme conjugate causing a color formation. The amount of color formed by a sample is then compared to the color formed by a PCP standard taken through all of the immunoassay steps, simultaneously. The comparison is made with the use of a differential photometer.

The system is portable and can be operated outdoors; however, temperature extremes and humidity can affect its performance. It is easy to use even by those with no immunoassay testing experience. The highest number of demonstration samples analyzed in one 10-hour day was 40; the average number analyzed in one 10-hour day was 23. The detection limit reported by the developer for soil samples is 0.5 ppm and that for water samples is 5 ppb. The system can be affected by naturally occurring matrix effects such as humic acids, pH, or salinity. Site-specific matrix effects that can affect the system include PCP carriers, such as petroleum hydrocarbons or solvents, and other chemicals used in conjunction with PCP, such as creosote, copper-chromium arsenate (CCA), or herbicides. Chemicals similar in structure to PCP can provide positive results. The system was found to be most affected by tetrachlorophenols and trichlorophenols. A specificity study performed during the demonstration showed that 2,3,4,6-tetra chlorophenol and 2,4,6-trichlorophenol would provide a positive response when present at a concentration of 10 ppm.

PRC evaluated field and laboratory duplicate samples to determine the technology's precision. The precision of the system for soil samples was found to be 79 percent. This is below the demonstration's criteria for acceptable precision. The precision of the system for water samples was found to be 100 percent. The comparison of the accuracy of the soil analysis showed that 73 percent of the time the technology was correct. The technology gave false positive results 19 percent of the time and gave false negative results 8 percent of the time. All of the false negative results were for samples containing less than 10 ppm. When examined for a PCP carrier effect, the frequency of correct readings was higher for the samples collected at the Winona Post site. The frequency of false positive and false negative results was similar between the two sites. The system produced correct results 47 percent of the time for the water analysis. It had a 42 percent false positive rate and an 11 percent false negative rate. When this data was examined for a PCP carrier effect, no false negatives were reported on samples from the former Koppers site; in addition the frequency of correct and false positive results was higher for the samples collected from this site.

Overall, the technology was found not to be accurate when compared to Level 3 data, but this technology can produce Level 2 data. However, in some cases it produced only Level 1 data. The technology is conservative; however, using an absolute definition of accuracy, it was accurate only 73 percent of the time. A draft version of this ITER was distributed on March 4, 1994. EnSys submitted no comments on the draft ITER.

Ohmicron Corporation: Penta RaPID Assay

The Penta RaPID Assay is designed to quickly provide quantitative results for PCP concentrations in soil and water samples. It uses immunoassay chemistry to produce compound-specific reactions to PCP allowing its detection and quantitation. Polyclonal antibodies are bound to paramagnetic particles and are introduced into a test tube where they offer binding sites for PCP. An enzyme conjugate containing a PCP derivative is added to

the test tube where it competes with PCP from samples for antibody binding sites. A magnetic field is applied to each test tube to hold the antibodies containing the PCP and enzyme conjugate, while excess sample and enzyme conjugate are removed from the test tube by washing. Reagents are then added to the test tube where they react with the enzyme conjugate causing a color formation. A solution is added to each test tube to stop color formation. The color formed by a sample is then compared to the color formed by three PCP standards taken through all of the immunoassay steps. The comparison is made with the use of a spectrophotometer.

The technology is portable, but should be used indoors because fluctuations and extremes in temperature or humidity may affect its performance. Also, the reagents require refrigeration, and the spectrophotometer requires electricity. The technology was found to be easy to operate by individuals with some prior analytical laboratory experience, but can be used by individuals with no prior immunoassay testing experience. The highest number of samples analyzed in one 10-hour day during the demonstration was 64, and the average number of samples analyzed in one 10-hour day was 21. The detection limit reported by the developer for soil samples is 0.1 ppm and for water samples is 0.06 ppb.

The technology may be affected by naturally occurring matrix effects such as humic acids, pH, or salinity. Site-specific matrix effects which can affect the technology include PCP carriers, such as petroleum hydrocarbons or solvents, other chemicals used in conjunction with PCP, such as creosote, CCA, or herbicides. Specific chemicals similar in structure to PCP can provide positive results with the technology. The technology was found to be most affected by tetrachlorophenols and trichlorophenols. A specificity study performed during the demonstration showed that 2,3,4,6-tetrachlorophenol and 2,4,6-trichlorophenol would provide a positive response when present at a concentration of 10 ppm.

PRC found no significant difference between the precision of the technology and that of the confirmatory laboratory. This conclusion was the same for both soil and water analysis. In addition, no PCP carrier effect on precision was observed. Results from the technology did not meet this demonstration's criteria for accuracy, as compared to Level 3 data. However, many of the data groupings produced were found to be linear, indicating that the results can be corrected mathematically. If 10 to 20 percent of the soil samples are sent to a confirmatory laboratory, then the results from the other 80 to 90 percent can be corrected. This need for mathematical correction to improve accuracy and the comparability of the technology's data with confirmatory data indicate that this technology can produce Level 2 analytical data. However, in some cases, this technology produced only Level 1 data. PRC found that the technology's water data was not significantly different from the confirmatory laboratory's when samples from the former Koppers site were analyzed. It indicated a significant difference between the data sets when the Winona Post site samples were analyzed. Therefore, the technology can produce Level 3 data for the former Koppers site water samples and Level 2 data for the Winona Post site water samples.

The developer submitted comments on a draft version of this report on May 4, 1994. These comments, which ranged from requests for clarification to technical comments on data interpretation, are available from EMSL-LV and PRC. In addition, the developer informed PRC that it now provides its customers with an "Environmental Users Guide," which advises them how to use the technology's quantitative data relative to action levels.

**Millipore Corporation:
EnviroGard PCP Test Kit**

The EnviroGard PCP Test Kit is designed to quickly provide semiquantitative or quantitative results for PCP concentrations in soil and water samples. Polyclonal antibodies are fixed to the inside wall of a test tube where they offer binding sites for PCP. An enzyme conjugate containing a PCP derivative is added to the test tube to compete with PCP from samples for antibody binding sites. Excess sample and enzyme conjugate is removed from the test tube by washing, and reagents are added to the test tube to react with the enzyme conjugate, causing color formation. The amount of color formed by a sample then is compared to the color formed by three PCP standards taken through all of the immunoassay steps. The results can be determined visually or a solution can be added to the test tube to stop color formation. A comparison of the sample and standards to a blank water sample is made with the use of a differential photometer. The differential photometer readings can be used to

provide semiquantitative results or a standard curve can be prepared to allow for a quantitative determination.

The developer was extremely concerned about the results of this demonstration, particularly the results showing the technology's tendency to produce false negative results when concentrations of PCP were greater than 1,000 ppm. The developer began an investigation into the causes of this tendency and has revised the technology as a result. The modifications included the addition of a detergent wash instead of a water wash. About one third of the samples analyzed during the demonstration were then reanalyzed by the developer. The developer has said the false positive and false negative rates then both fell to 3 percent. The SITE Program, though, has not demonstrated and evaluated the technology as modified by the developer. All of the modifications have been incorporated into the technology's commercial product. The developer said in its letter to EPA regarding this demonstration: "We believe that, based on the knowledge gained from the field demonstration, we have been able to make significant improvements in our product. This experience demonstrates the utility of the SITE program and its goal of evaluating innovative technologies."