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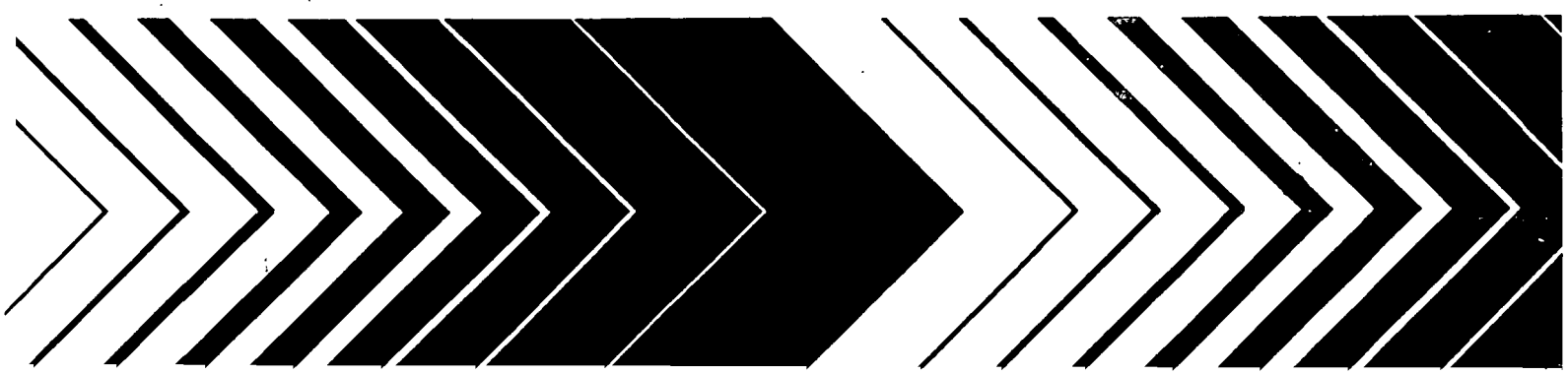
Superfund Innovative Technology Evaluation (SITE) Report for the Westinghouse Bio-Analytic Systems Pentachlorophenol (PCP) Immunoassays



SUPERFUND INNOVATIVE TECHNOLOGY EVALUATION (SITE) REPORT FOR THE WESTINGHOUSE BIO-ANALYTIC SYSTEMS PENTACHLOROPHENOL (PCP) IMMUNOASSAYS

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ABSTRACT

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.

The demonstration was designed to evaluate the ruggedness and utility of a semiquantitative immunoassay field kit. The 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 demonstration was performed at the MacGillis & Gibbs Superfund Site in New Brighton, Minnesota, a National Priorities List site known to have ground water contaminated with pentachlorophenol. The immunoassay demonstration was conducted jointly with a SITE demonstration of a bioremediation technology. This technology was designed by BioTrol, Inc. (Chaska, Minnesota), to biodegrade pentachlorophenol in aqueous matrixes and waste streams.

The results of the WBAS immunoassay demonstration support the conclusion that the field immunoassay is a useful screening tool. Though the study's data quality objectives for accuracy and precision were only partially met, most of the results were close to the expected concentrations. Results 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 with a bias toward a high concentration when compared to GC/MS, 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 evaluation is submitted in partial fulfillment of Contract Nos. 68-03-3249 and 68-CO-0049 by Lockheed Engineering & Sciences Company under the sponsorship of the EPA.

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FIGURES (continued)

TABLES

ABBREVIATIONS AND ACRONYMS

AFMMP	Advance Field Monitoring Methods Program
ANOVA	analysis of variance
BATS	Biological Aqueous Treatment System
CLP	Contract Laboratory Program
CV	coefficient of variation
DNP	dinitrophenol
DQO	data quality objective
EMSL-LV	Environmental Monitoring Systems Laboratory-Las Vegas
EPA	U.S. Environmental Protection Agency
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GLP	good laboratory practice
gpm	gallons per minute
L	liter
LESC	Lockheed Engineering & Sciences Company
mL	milliliter
MMTP	Monitoring and Measurement Technologies Program
n	number
N	normal
NC	negative control
nm	nanometer
OD	optical density
OMMSQA	Office of Modeling, Monitoring Systems and Quality Assurance
PAH	polynuclear aromatic hydrocarbon
PCP	pentachlorophenol
pH	negative log of the hydrogen ion activity
ppb	parts per billion
ppm	parts per million
QA	quality assurance
QAA	quality assurance performance sample, type A
QAB	quality assurance performance sample, type B
QAPjP	quality assurance project plan
QC	quality control
RREL	Risk Reduction Engineering Laboratory
SAIC	Science Applications International Corporation
SARA	Superfund Amendments and Reauthorization Act
SAS	Statistical Analysis System
SITE	Superfund Innovative Technology Evaluation
SOP	standard operating procedure
WBAS	Westinghouse Bio-Analytic Systems

ACKNOWLEDGEMENTS

This demonstration and the subsequent preparation of this report required the services of personnel from the U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory (Las Vegas, Nevada); the U.S. Environmental Protection Agency, Risk Reduction Engineering Laboratory (Cincinnati, Ohio); Westinghouse Bio-Analytic Systems (Rockville, Maryland); Science Applications International Company (Paramus, New Jersey; San Diego, California; and McLean, Virginia); BioTrol Inc., (Chaska, Minnesota); and Lockheed Engineering & Sciences Company (Las Vegas, Nevada). Names of individuals and their functional roles on behalf of these organizations are included in Appendix E.

EXECUTIVE SUMMARY

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).

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

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, and 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 percent) of the GC/MS results. Traditional methods such as GC/MS have interlaboratory biases of 30 percent 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 usable) 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.

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.

Results from the on-site kit immunoassay compared favorably to the GC/MS results. 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 percent 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 percent) 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 percent) 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 immunoassay, the plate immunoassay exhibited a high bias when compared to the GC/MS, although the bias was much smaller (17 to 40 percent for influent samples, depending on analysis site).

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 percent), 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 percent 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 percent), 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.

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 throughput 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.

SECTION 1

INTRODUCTION

The performance of two immunoassay methods was assessed during a U.S. Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation (SITE) Program demonstration. The methods are semiquantitative and quantitative immunoassay techniques developed by Westinghouse Bio-Analytic Systems (WBAS) of Rockville, Maryland, to detect pentachlorophenol (PCP) in water. The immunoassays were demonstrated under field (on site) and laboratory (off site) conditions and were compared to a standard EPA gas chromatography/mass spectrometry (GC/MS) method.

OVERVIEW OF THE IMMUNOASSAY PROGRAM

The EPA Environmental Monitoring Systems Laboratory at Las Vegas, Nevada (EMSL-LV), is responsible for developing and evaluating immunoassays for specific environmental applications. According to EPA guidelines for methods evaluations, this process requires the determination of performance parameters such as precision, within- and among-laboratory biases, between-method bias, method detection limits, interferences, and ruggedness of the method.

To be effective as rapid screening tools, immunoassays must provide timely, cost-effective results that complement conventional analytical methods. They must be capable of measuring the target analyte with sufficient accuracy and precision to identify that samples are clearly above or below a critical concentration range.

OVERVIEW OF THE SITE PROGRAM

The Superfund Amendments and Reauthorization Act of 1986 (SARA) mandated that the EPA develop timely and cost-effective remedies at National Priorities List (i.e., Superfund) sites. As part of the response to this mandate, the EPA established SITE, "a program of research, evaluation, testing, development, and demonstration of alternative or innovative treatment technologies....which may be utilized in response actions to achieve more permanent protection of human health and welfare and the environment" (SARA, 1986). The SITE Program is comprised of two innovative technology categories. The first category includes the demonstration of alternative treatment technologies that can be used in Superfund site remediation. These activities are administered by the EPA Risk Reduction Engineering Laboratory (RREL) at Cincinnati, Ohio. The second category is for the evaluation of measurement and monitoring techniques that can withstand the rigors of field conditions. This portion of the SITE Program, the Monitoring and Measurement Technologies Program (MMTP), is administered by the Advanced Field Monitoring Methods Program (AFMMP) of the EPA Office of Modeling, Monitoring Systems, and Quality Assurance (OMMSQA). The Environmental Monitoring Systems Laboratory at Las Vegas, Nevada, is the lead laboratory for the

AFMMP. The immunoassay procedures evaluated in this report represent the first measurement and monitoring technologies to be evaluated under MMTP in the SITE Program.

OVERVIEW OF THE WBAS SITE DEMONSTRATION

The WBAS SITE demonstration was conducted primarily to assess the ruggedness and utility of a semiquantitative immunoassay field analysis technique for rapid sample screening. The demonstration also provided the opportunity to further evaluate a quantitative 96-well, microtiter plate immunoassay (see Section 2 for a discussion of the previous evaluation). The two immunoassay methods were developed by WBAS to detect PCP in water under field and laboratory conditions. These techniques, referred to in this report as "kit immunoassay" and "plate immunoassay," respectively, were compared to each other and to a standard EPA GC/MS method for detecting PCP in water.

The demonstration took place from July 23 to August 29, 1989, at the MacGillis & Gibbs Superfund Site at New Brighton, Minnesota. This location was well suited for the evaluation of the WBAS immunoassays for several reasons. Ground water at the site was contaminated with PCP and polynuclear aromatic hydrocarbons (PAHs) as the result of a wood preservative treatment operation. In addition, a SITE demonstration at the MacGillis & Gibbs site had been previously planned for the summer of 1989. RREL was demonstrating the BioTrol Aqueous Treatment System (BioTrol, Inc., Chaska, Minnesota), a biological reactor (bioreactor) designed to biodegrade PCP and PAHs in aqueous media into carbon dioxide, water, and sodium chloride (Stinson et al., 1991, and Appendix A). In an effort to minimize complications involved in adding a design to one that was already in place, the sampling and analysis scheme of the WBAS immunoassay demonstration was constructed around the design of the bioreactor demonstration.

PROJECT ORGANIZATION

The success of the immunoassay evaluation depended on the coordinated efforts of four primary organizations: EMSL-LV, WBAS, RREL, and BioTrol, Inc.

EMSL-LV, with assistance from its contractor, Lockheed Engineering & Sciences Company (LESC), was responsible for:

- Designing, overseeing, and ensuring the implementation of the elements of the demonstration and quality assurance (QA) plan.
- Acquiring the necessary confirmatory data.
- Performing off-site analysis by both immunoassay techniques and by GC/MS.
- Preparing and distributing QA and quality control (QC) samples.
- Evaluating and reporting on the performance of the technologies.

WBAS, the developer of the immunoassays, was responsible for:

- Performing preliminary testing to assess kit immunoassay performance.

- Supplying a sufficient number of field kits and plate immunoassay reagents to perform the analyses required to conduct the demonstration.

- Providing technical assistance to the on-site personnel using the kit immunoassay.

- Performing off-site analyses by both immunoassay techniques.

RREL, through its contractor, Science Applications International Corporation (SAIC), Paramus, New Jersey, conducted the BioTrol, Inc., bioreactor demonstration. RREL and SAIC were responsible for the following aspects of the immunoassay demonstration:

- Performing the on-site kit immunoassay analysis on designated samples.

- Providing logistical support, including field sample collection, processing, and shipment.

- Analyzing samples by GC/MS and reporting the results.

For the immunoassay demonstration, BioTrol, Inc., was responsible for:

- Providing predemonstration test samples.

- Providing technical assistance.

Figure 1 shows the organizational structure of the immunoassay demonstration.

SCOPE OF DOCUMENT

This document includes descriptions of the methods and operating theories of the two WBAS immunoassay technologies (Section 2), designs of the SITE demonstration and QA plans (Section 3), and comparisons of the immunoassay methods to a standard EPA method (Section 4). The results of intra- and intermethod and QA/QC performances are described in Section 5. Conclusions and recommendations about the WBAS immunoassay technologies are discussed in Section 6.

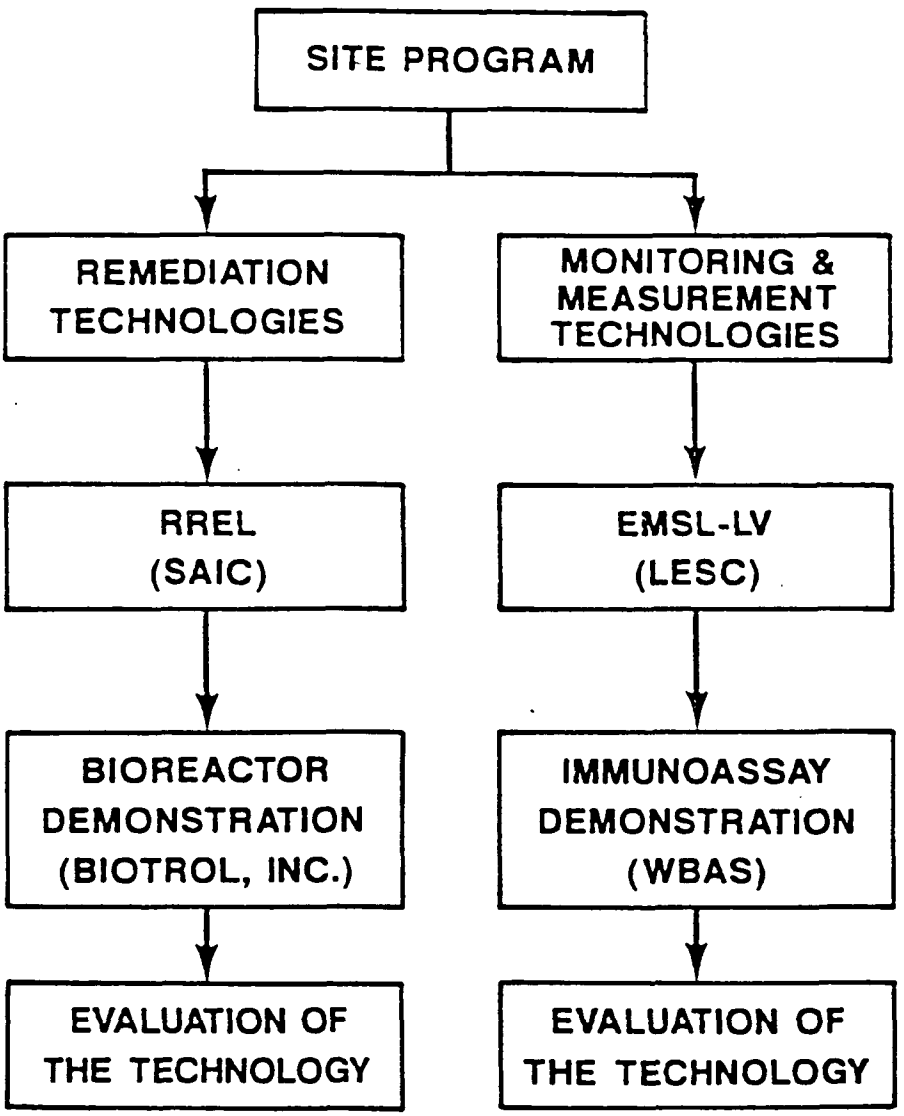

Figure 1. Organizational structure for the SITE demonstration of the WBAS immunoassays at the MacGillis & Gibbs Superfund Site.

SECTION 2

DESCRIPTIONS OF TECHNOLOGIES

Immunoassays are analytical techniques based on protein molecules called antibodies. The binding of a specific antibody to its target analyte can be used to detect or quantitate contamination in environmental samples. Specific antibodies can be developed to detect either a single analyte (e.g., compound) or groups of related compounds. Plate immunoassays have a high sample capacity, are quantitative in nature, and may take 3 to 4 hours of preparation and analysis time to run. Kit immunoassays, on the other hand, are usually designed for rapid qualitative or semiquantitative on-site measurements. Both immunoassay technologies are portable, though plate immunoassays may require more logistical and equipment considerations for use in the field. Immunoassay techniques are quite versatile and can be applied to many analytical situations. The WBAS kit and plate immunoassay techniques and their theories of operation are discussed in the following sections.

WBAS KIT IMMUNOASSAY

The WBAS immunoassay kit (Figure 2) is designed for on-site qualitative or semiquantitative determination of PCP in water. In this evaluation, the kit immunoassay was used to provide semiquantitative results. The test, which is based on rabbit polyclonal antisera, has a reported detection limit of 3 parts per billion (ppb) and a total analysis time of approximately 30 minutes. The method has a linear dynamic range of 3 to 40 ppb.

The kit immunoassay is performed in an 8-well, polystyrene, microtiter strip that is coated with anti-PCP rabbit polyclonal antibody. Each well has a volume capacity of approximately 0.25 mL. Figure 3 depicts the steps required for analysis by the kit immunoassay. Calibration standards of 3.0, 7.1, 16.9, and 40.0 ppb PCP are used in four of the eight wells. Environmental water and QA/QC samples are diluted into the range (i.e., 3 to 40 ppb) for quantitation estimates in the four remaining wells. Fifty μL of sample or standard are placed in a well. Fifty μL of enzyme-labeled PCP analog (PCP-Peroxidase) are then added. Competition between PCP in the sample and PCP-Peroxidase occurs for binding to the immobilized antibody in the well. After a 15-minute incubation, all unbound materials are removed by washing with a buffer rinse. Next, 100 μL of a 1:1 mixture of an enzyme substrate (H_2O_2) and chromogen ($3,3^1,5,5^1$-tetramethylbenzidine) are added. The immobilized enzyme acts on these substances, producing a colored end product. Fifty μL of 0.3 N sulfuric acid are added to stop further color production. A portable spectrometer (manufactured by Hyperion, Inc., for Dynatech Laboratories, Inc.)(Figure 4), is used to measure the optical density (OD) of the colored end product at 405 nm (Figure 4). Since the quantitation is based on competition for antibody, the color intensity is inversely proportional to the analyte concentration in the sample. Quantitation of PCP is determined by using a standard curve. These estimates can be determined by manually plotting the four calibration standards on semi-log graph paper, or the standard curve can be fitted by a best-fit

line generated with a programmable calculator. Examples of hand-plotted standard curves for the kit immunoassay are given in Appendix B.

WBAS PLATE IMMUNOASSAY

The WBAS plate immunoassay for quantitation of PCP in water samples is also a competitive inhibition enzyme immunoassay. A 96-well, polystyrene, microtiter plate coated with 2,6-dichlorophenol-protein (DCP-protein) conjugate is used for the solid phase. The free analyte in the sample and the bound conjugate compete for binding to the anti-PCP antibody in solution. An enzyme-labeled secondary antibody is used to quantitate the amount of anti-PCP antibody bound to the 2,6-dichlorophenol-protein conjugate on the solid phase. The amount of bound anti-PCP

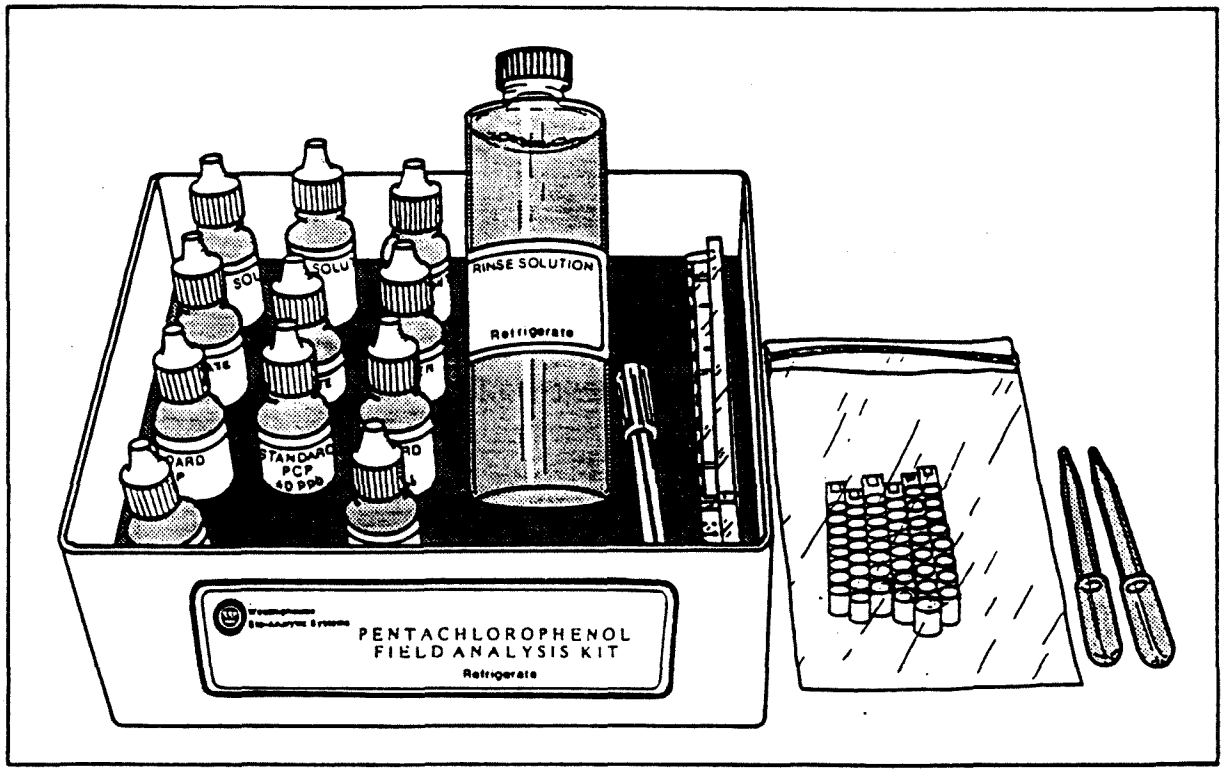

Figure 2. WBAS field kit immunoassay.

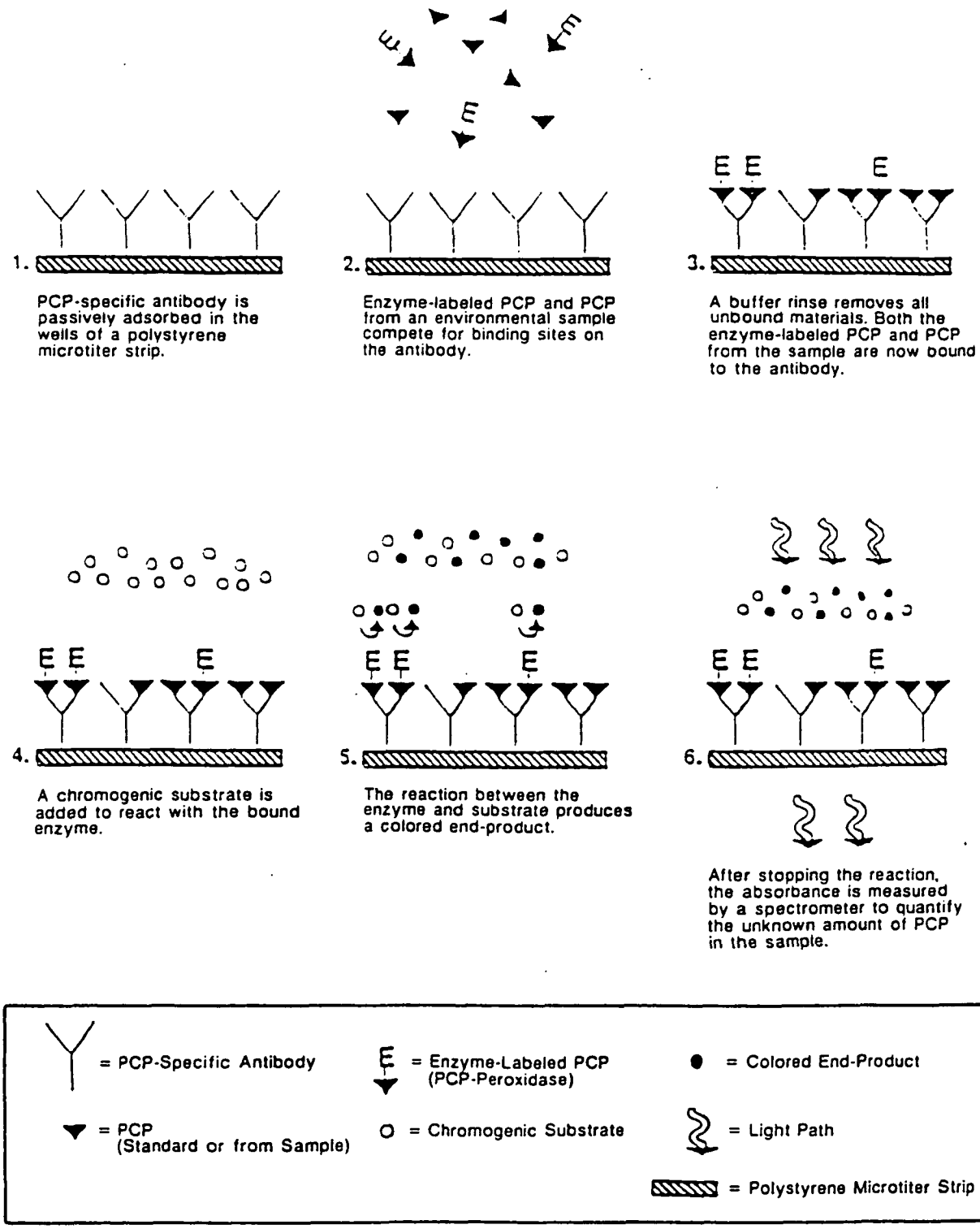

Figure 3. WBAS kit immunoassay analysis steps for determining pentachlorophenol in aqueous samples.

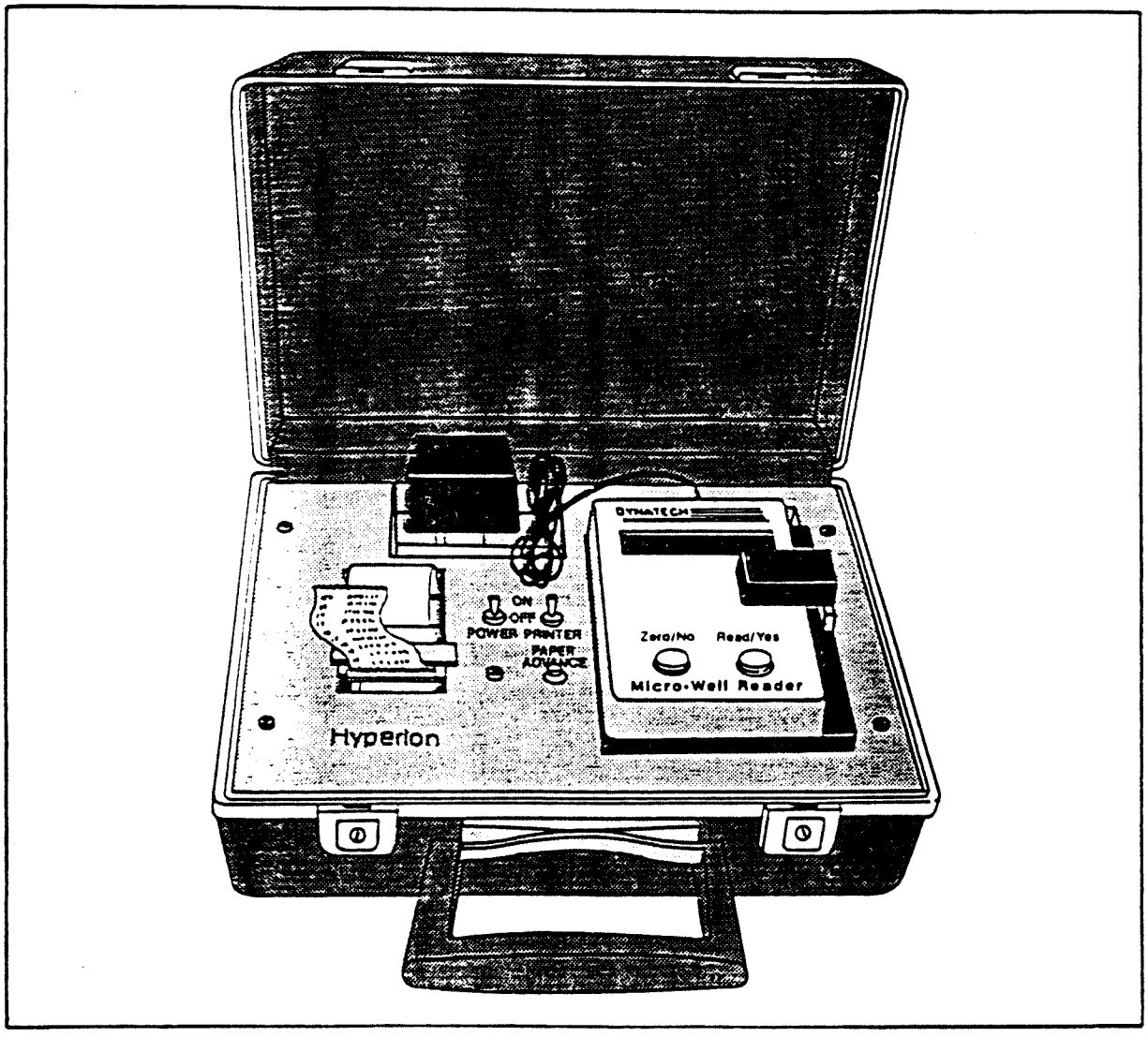

Figure 4. Portable spectrometer used for the WBAS kit immunoassay demonstration.

antibody is inversely related to the amount of PCP in the sample. In the final procedural step, an enzyme substrate is added, and the colored end product OD is read with a plate reader (HP/Genenchem®) at 405 nm. The intensity of color is inversely proportional to the concentration of analyte in the sample. Figure 5 shows the analysis steps described above for the plate immunoassay.

The plate immunoassay has a detection level of approximately 30 ppb PCP and a linear dynamic range from 50 to 400 ppb. If the extraction procedure in EPA Method 604 (U.S. EPA, 1982) is used on the water samples, the minimum detectable level is below 1 ppb. The plate immunoassay procedure involves an overnight incubation step to generate a plate coating (needing about 10 minutes of the analyst's time); however, the actual analysis time is only about 3 hours. The method has a high sample throughput because 10 to 20 samples can be run in triplicate on each plate, and several plates can be run in batches in one day. An example of the sample placement layout on a typical plate analyzed in the SITE evaluation is given in Appendix B.

Results from the 96-well plate immunoassay are calculated using a 4-parameter curve fit generated by the TiterCalc® data analysis program. Results were quantitated using the approximately linear region of the standard curve (i.e., the region between 10 percent and 90 percent OD range). An example of a typical standard curve is given in Appendix B. Data generated by EMSL-LV and WBAS from the plate immunoassay were collected in hard copy form and on floppy diskettes using the TiterCalc® software.

Previous Plate Immunoassay Laboratory Evaluation

A methods comparison was conducted at EMSL-LV prior to the SITE demonstration (Van Emon and Gerlach, 1990) comparing the plate immunoassay and a gas chromatography (GC) detection protocol (EPA Method 604 [U.S. EPA, 1982]). This study used environmental water samples (i.e., drinking water, surface water, and ground water) spiked with PCP at various concentrations to evaluate the technology. Extracts were prepared following protocols in EPA Method 604 and quantitated by both the plate immunoassay and GC for comparison. Extracts from a simple solid-phase extraction technique, developed by WBAS, were also analyzed by the plate immunoassay and by GC. In addition, unextracted samples were analyzed directly by the plate immunoassay (direct plate immunoassay) as the method does not require an extraction for relatively clean samples (i.e., not silty). The direct plate immunoassay data were compared to the GC results obtained with the solid-phase and EPA Method 604 extracts.

The results of this previous evaluation showed no practical difference between: (1) the plate immunoassay and GC detection of Method 604 extracts, (2) the plate immunoassay and GC detection of solid-phase extracts, (3) immunoassay results from WBAS and EMSL-LV, following the WBAS solid-phase or EPA Method 604 extraction protocols, and (4) precision of the direct plate immunoassay obtained by WBAS and EMSL-LV. Overall, this study generated a 9 percent false positive rate (based on blank sample analysis; n = 115) and a 0 percent false negative rate (based on spiked environmental water samples; n = 192). It is important to emphasize that the plate immunoassay could be performed directly on the environmmental water samples without an extraction, although the direct method had a higher variability than immunoassay following either extraction technique. Thus, the method could be performed in a field laboratory as a quantitative, high-sample capacity, analytical methodology.

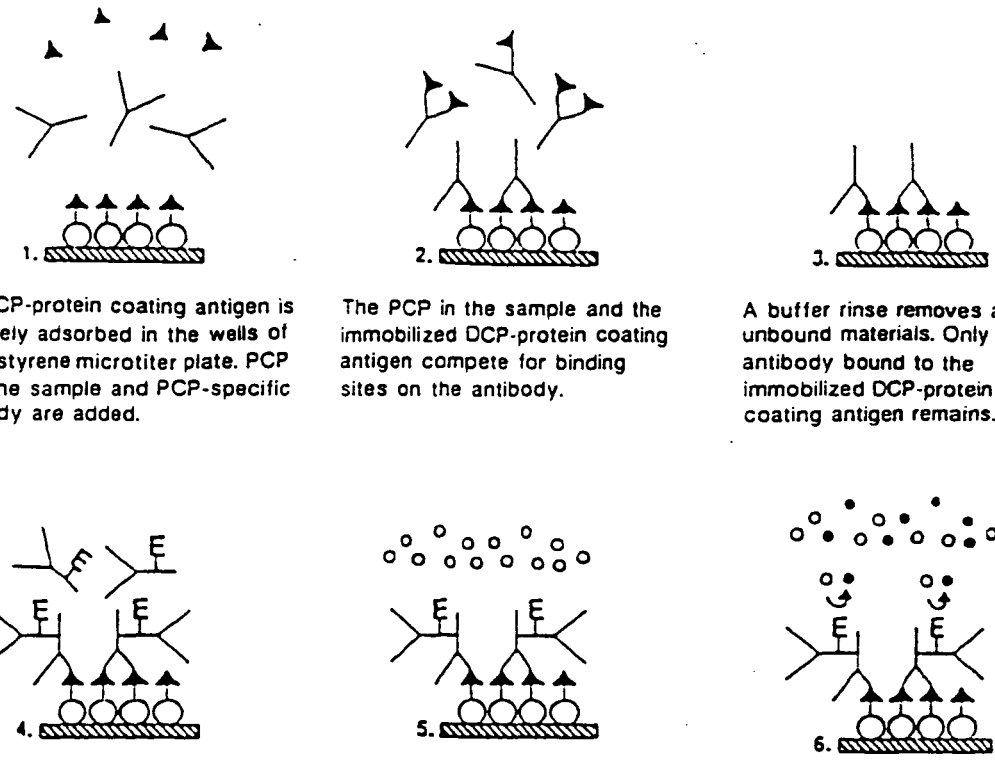

Figure 5: WBAS plate immunoassay analysis steps for determining pentachlorophenol in aqueous samples.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS

The GC/MS method used in the WBAS immunoassay SITE demonstration was performed in two main steps: (1) sample preparation and (2) analysis by GC/MS. Samples were liquid extracted according to EPA Method 3510 (OSWER, 1986), a procedure for isolating and concentrating organic compounds from aqueous samples. Sample extracts were then analyzed using EPA Method 8270 (OSWER, 1986), a GC/MS method for semivolatile organics, including PCP and PAHs. This method is designed for analysis of extracts prepared from all types of solid waste, soil, and ground-water matrices. Method 8270 contains detailed analysis instructions, QC guidelines, and performance data for PCP analysis by GC/MS. The practical quantitation limit of the method is 50 ppb for PCP in ground water. In addition, the method states that the experience of the analyst performing the GC/MS analyses is invaluable to the success of the method (OSWER, 1986). These methods were chosen as the most appropriate for the goals of the bioreactor demonstration because a wide spectrum of PAHs were of interest in addition to PCP. This particular GC/MS method was one of several valid EPA techniques available for the analysis for PCP.

SECTION 3

PCP IMMUNOASSAY DEMONSTRATION DESIGN

By conducting the immunoassay demonstration in conjunction with the BioTrol, Inc., bioreactor demonstration, the EPA was presented with an excellent opportunity to simultaneously test the effectiveness of remediation and monitoring and measurement technologies. The bioreactor demonstration had been in the negotiation and planning stages for a SITE demonstration far in advance of the plan to evaluate the immunoassay. In an effort to minimize complications, the sampling and analysis scheme of the WBAS immunoassay demonstration was constructed around the activities planned for the bioreactor demonstration.

The design of the SITE demonstration to evaluate the capabilities and limitations of the WBAS kit and plate immunoassays included several components. Predemonstration testing provided insights that were incorporated into the final design. The immunoassay sampling and QA plans were structured to ensure the collection of enough data to make the necessary method and statistical comparisons and to assess the quality of the data. In addition, a data management system was developed to ensure reliable collection, integration, analysis, presentation, and storage of the data generated during this SITE demonstration.

PREDEMONSTRATION TESTING AND PLANNING

From late May through mid July of 1989, WBAS conducted a variety of preliminary performance checks on the kit immunoassay in a controlled laboratory environment. The preliminary checks were valuable in finalizing the overall design of the immunoassay demonstration. Performance data were generated using specified concentrations of PCP spiked into laboratory-grade water, ground water, and bioreactor-matrix water samples. The ground-water and bioreactor samples were provided to WBAS by BioTrol, Inc. These samples were not collected from the MacGillis & Gibbs site, but were used to simulate samples that would be obtained during the demonstration.

The predemonstration tests provided insight into such operating and data quality parameters as: linear dynamic range of the calibration curve; matrix effects and interferences, especially for the effluent samples; QC checks (e.g., sample dilution, pretreatment, and procedural precautions); estimates of precision from replicate and dilution analyses; bias resulting from different spectrometers; and the stability of PCP in the field samples. The conclusions obtained from the predemonstration tests are presented below.

- **Cross-reactivity**--A variety of structurally related compounds were tested for their cross-reactivity in the plate and kit immunoassays. The results are summarized in Table 1. For the plate immunoassay, a 42 percent response relative to PCP was found for 2,3,5,6-tetrachlorophenol, and two trichlorophenols yielded about a 10 percent relative response.

Other tested compounds gave much less or negligible responses. Similarly, the highest cross-reactivity for the kit immunoassay was 19 percent for 2,3,5,6-tetrachlorophenol. Several trichlorophenols showed cross-reactivities in a range of 7 to 11 percent. As with the plate immunoassay, much less cross-reactivity was observed for the other tested compounds.

TABLE 1. CROSS-REACTIVITY OF ANTI-PENTACHLOROPHENOL ANTIBODIES

	Percent cross-reactivity[a]	
Compound	Plate	Kit
2,3,5,6-Tetrachlorophenol	42.0	19
2,4,6-Trichlorophenol	12.0	7
2,3,6-Trichlorophenol	8.8	7
2,6-Dichlorophenol	1.8	0.4
Tetrachlorohydroquinone	0.8	0.7
2,3,4-Trichlorophenol	0.5	11.0
2,3,5-Trichlorophenol	0.5	2.5
2,4-Dichlorophenol	0	N/A
2,5-Dichlorophenol	0	0.1
3,5-Dichlorophenol	0	0.1
3,4-Dichlorophenol	0	0.1
2,3-Dichlorophenol	0	0.1
4-Chlorophenol	0	0.2
Phenol	0	0.1
Pentachloroaniline	0	0.1
Pentachlorobenzene	0	N/A
2,3-Dinitrotoluene	0	0.1
2,4-Dinitrotoluene	0	0.1
2,4,5-Trichloronitrobenzene	0	0.1

[a][IC_{50}PCP/IC_{50} compound] x 100, where IC_{50} is the molar concentration of compound that inhibits 50 percent antibody binding in immunoassays.

Source: Courtesy of WBAS

- **Calibration standards**--A series of calibration standards were used to evaluate the linear dynamic range of the kit. Analysis of the standards revealed that the linear part of the curve was from 3 to 40 ppb instead of the 3 to 100 ppb range that was originally indicated by WBAS. The narrowing of the linear range changed a variety of design components, including matrix spiking concentrations, sample dilution schemes, and protocol specifications.

- **Replicate analyses**--Replicate analyses (n = 12) of 4 ppb and 30 ppb PCP standards, representing the low and high portions of the linear dynamic range, were used to evaluate kit immunoassay accuracy and precision. The 4 ppb standard yielded a mean concentration of 5.2 ppb (15 percent coefficient of variation [CV]), and the 30 ppb standard yielded a mean concentration of 44 ppb (36 percent CV). The results of these analyses indicated the potential for a high bias.

- **Holding time study**--Results of a holding time study, which involved the storage of splits of influent and effluent samples for 6 days at room temperature and at 4 °C, indicated that changes caused by this temperature storage differential were negligible.

- **Range-finding and dilution checks**--Field samples were diluted in order to evaluate the expected range of PCP concentrations. Three serial dilutions were made of an influent sample containing approximately 18 ppm PCP so that the dilution results fell on three areas in the linear calibration range. The assessment of the results of these range-finding and dilution checks proved useful in assessing the approximate dilution factors to be used during the demonstration.

- **Pipetting Accuracy**--WBAS discovered a high degree of inaccuracy in the squeeze dropper bottles that were supplied with the kit immunoassay. To rectify the problem, WBAS suggested the use of a mechanical pipettor (Rainin®) in the kit analysis.

- **Blank samples**--Blank, or negative control, samples distributed over 12 strips were used to estimate solid phase variability (i.e., the variability among microtiter wells and strips). Results indicated that there was approximately an 11 percent CV on these measurements.

- **Matrix spike analyses**--On two separate days, matrix spike analyses were performed on four effluent samples diluted to 10 ppb and spiked with 15 ppb PCP. This spike level was chosen so that all samples would fall within the linear range of the method. The mean recoveries of the analyses for each day were 163 percent and 120 percent, respectively. These results indicated a potential problem with matrix spike analysis for the kit immunoassay, and problems with matrix spike analysis were encountered in the demonstration (see Section 5).

Based on the above predemonstration data and on verbal and written information provided by WBAS, RREL, SAIC, and BioTrol, Inc., EMSL-LV developed demonstration and QA plans (Silverstein et al., 1991) and field instructions. EMSL-LV also prepared, verified, and distributed all QA and QC sample materials. WBAS conducted a 1-day training session for the SAIC field personnel designated to perform the on-site kit immunoassay analyses.

STUDY DESIGN FOR THE SITE DEMONSTRATION AND EVALUATION

The field samples for the immunoassay SITE demonstration were collected in three 1-week (6-day) intervals, alternating weekly for a period of 6 weeks. Samples were collected and split on site; one split was analyzed at the field site by the kit immunoassay. The on-site analyses were performed in a field trailer at the MacGillis & Gibbs site. This trailer was also used for various sample preparation and shipment activities for both the immunoassay and bioreactor demonstrations. Split samples were shipped to the EMSL-LV and WBAS laboratories for analysis by both the kit and plate immunoassay methods. Sample splits were also sent for analysis by GC/MS at the SAIC laboratory in San Diego, California. In addition, a selected group of samples was analyzed by GC/MS at the EMSL-LV facility. EPA Method 8270 (OSWER, 1986), after sample extraction by EPA Method 3510 (OSWER, 1986), was used as the confirmatory and comparative method. The sample flow and analysis scheme is presented in Figure 6.

The MacGillis & Gibbs site was ideal for the application of the immunoassay technologies because the ground water was known to contain high levels of PCP (approximately 50 ppm). The ground water was also relatively clean, requiring no cleanup before entering the bioreactor. Although some samples were turbid, colored yellow or brown, or had a precipitate, no solid phase or centrifugation cleanup was required for the immunoassay analyses. The bioreactor was operational for 6 weeks, which were divided into three 2-week periods that coincided with changes in flow rate of the ground water pumped through the bioreactor. Flow rates of 1, 3, and 5 gallons per minute (gpm), or periods A, B, and C, respectively, delineated these 2-week periods. Samples were collected for the immunoassay demonstration 6 days within each flow period (18 total days) in order to get a diverse set of field samples while minimizing logistical effects on the bioreactor SITE demonstration activities. The EMSL-LV and WBAS laboratories only analyzed samples from the first and third (A and C) weeks of the demonstration by kit immunoassay. There was a concern regarding a potential shortage of reagents due to the short lead time of the study. As a result, about one-third fewer samples were available for comparison. For various logistical reasons, not all of the analysis sites analyzed all samples. However, enough samples were analyzed by each method and at each site to conduct the necessary method comparisons between the kit immunoassay, the plate immunoassay, and the established GC/MS method (see sections 4 and 5).

The PCP-contaminated ground water was pumped into the bioreactor and discharged as treated effluent. Data from the site characterization showed that the raw ground water contained approximately 50 ppm PCP. Preliminary data from the bioreactor indicated the treated effluent would contain 1 ppm or less. Two daily sampling points were selected at the entry and exit points of the bioreactor. A diagram of the bioreactor and the sampling points is shown in Figure 2 of Appendix A. The first daily sampling point is denoted as "No. 2" in the figure. Samples collected at this point consisted of ground water, which had been conditioned by pH adjustment with NaOH (to an approximate pH of 7.3) and nutrient addition (nitrogen and phosphorous compounds) to enhance bacterial growth inside the bioreactor. The samples collected at the second daily sampling point, "No. 5" in Figure 2 of Appendix A, consisted of bioreactor effluent before carbon filtration and removal of solids. For this report, these samples are termed "influent" and "effluent" samples, respectively.

Many of the effluent samples were somewhat turbid, presumably as a result of the biological and chemical processes and constituents inside the bioreactor. Centrifugation of effluent samples was considered in the original design of the demonstration, but this option was abandoned in order to avoid bias between the immunoassay and the GC/MS results. In addition, a weekly grab well water sample, termed "raw influent," was collected (i.e., prior to pH and nutrient conditioning). The raw influent sample was collected in bulk as a grab sample from a T-tap located forward of the conditioning tank (sampling point "No. 1", Figure 2 of Appendix A). A total of 18 influent, 18 effluent, and 3 raw influent bioreactor samples were collected for the immunoassay demonstration. In addition, 18 field blank samples were analyzed to evaluate the effectiveness of the decontamination method for the sample collection apparatus and vessels.

Sample Collection Procedures

The influent and effluent samples were collected with separate automatic composite sampling devices in 150-mL portions every 20 minutes over a 24-hour period. After the bulk sample (approximately 13 L) was collected, it was split into homogenous subsamples for on-site analysis and shipment to the EMSL-LV, WBAS, and SAIC laboratories. In addition, field blank samples were collected after daily

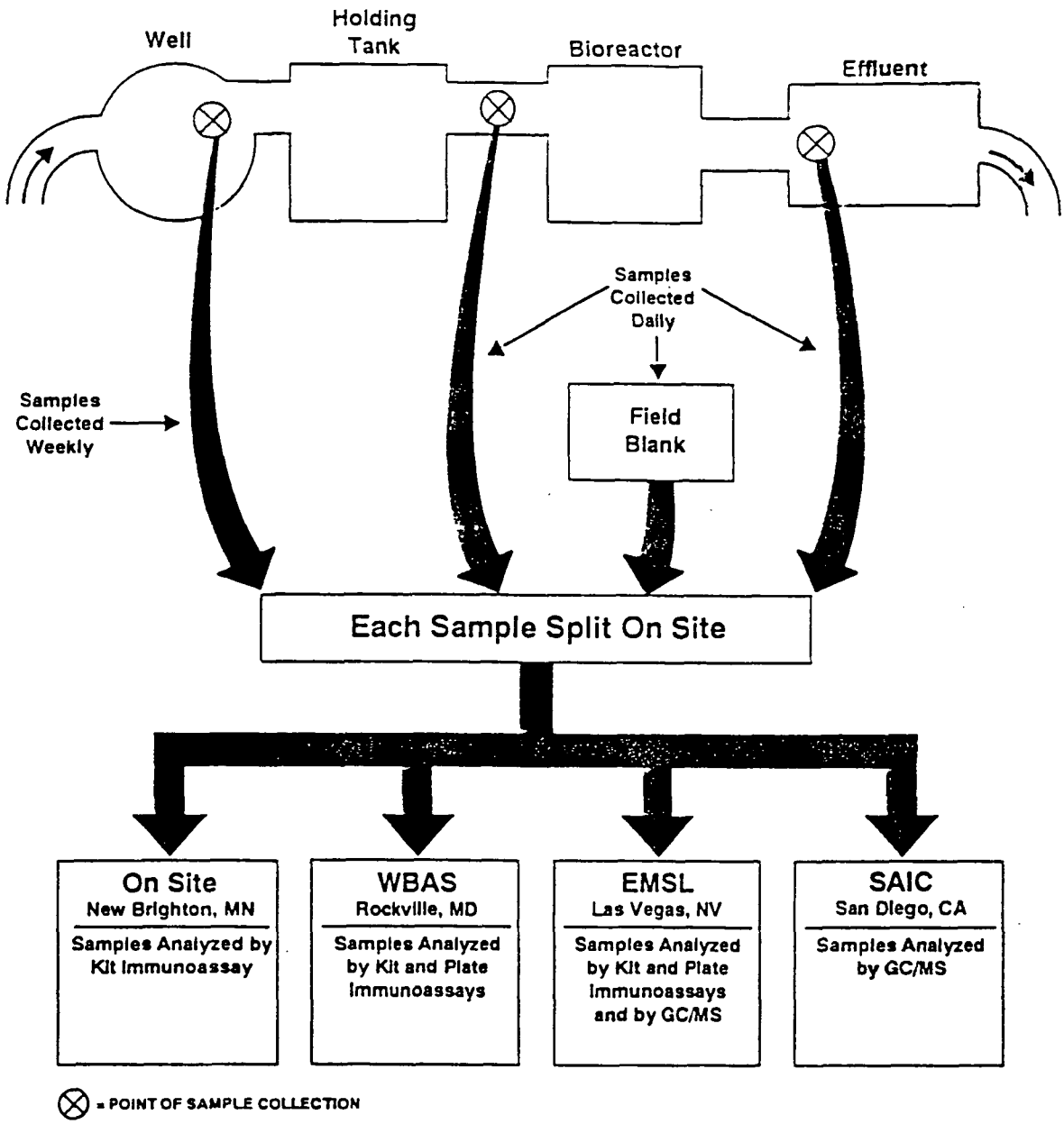

Figure 6. Sample flow and analysis scheme for the SITE demonstration of the WBAS immunoassays for pentachlorophenol.

decontamination of the composite sampler. They consisted of reagent-grade water poured into a clean (i.e., decontaminated) composite jug, pumped through the sampler and collected directly into the appropriate bottles. Field blanks were collected at influent and effluent sampling points on alternate days.

Samples collected for immunoassay analyses were prepared in the same manner as the samples for GC/MS analysis, except a smaller volume was collected for immunoassay. Sample splits were prepared in amber glass bottles with Teflon-lined, screw caps in 30-mL, 250-mL, and 4-L volumes for the kit immunoassay, plate immunoassay, and GC/MS analyses, respectively. All samples were stored at 4 °C prior to on-site analysis or shipment. Field samples were packed with custody tape wrapped around the neck and cap of each container, wrapped with insulation, and shipped in coolers maintained at 4 °C. To maintain a record of sample collection, transfer, shipment, and receipt, a chain-of-custody form was filled out for each shipment to each analysis location. In addition, EMSL-LV prepared and shipped semiblind (audit) and known (QC) performance samples and matrix spiking solutions to the field and the off-site laboratories.

Sample Tracking

Sample tracking was accomplished by assigning each sample a unique identification number as it was collected. This number traced the sample day, time, and point of collection. Along with other information on the label, the sample number provided the tracking information for samples analyzed on site and off site. This numbering and documentation system proved invaluable when verifying data after the field operations were completed. Because of the complexity of the study and QA designs of the demonstration, a series of sample codes were also used to identify the samples for the purpose of assessing data quality (Silverstein et al., 1991).

QUALITY ASSURANCE DESIGN

QA planning was a critical element of the study design. To ensure that data from this demonstration were of known quality and representative of typical conditions, a QA project plan (QAPjP) was prepared and followed. The QAPjP addressed the key elements required for Category II projects (U.S. EPA, 1987) and enabled analysts to make data quality and performance estimates, conclusions, and recommendations. Sections 4, 5, and 6 present these results.

Influent and effluent samples provided valuable QA data because they were homogenous subsample splits (field splits) that were sent to each analysis site. A variety of QA and QC samples were also used in this demonstration. These are described below.

- **Duplicate and method split samples**--Duplicate samples are defined in this study as bioreactor samples that were divided into two separate subsamples at an analysis site prior to being diluted into calibration range for analysis. Method split samples are defined as bioreactor samples that are first diluted into calibration range and then split for analysis. The purpose of these samples was to assess the variability associated with sample dilution efficiency (with the duplicate samples) as compared to the variability associated with analyzing an already diluted sample (with the method split samples) in two separate wells (for the kit immunoassay) or sets of wells (for the plate immunoassay). (NOTE: The

above method splits should not be confused with the field splits, which were subsamples of the original bulk sample.)

- **Matrix spikes**--Matrix spike analysis consists of adding a known quantity of analyte to a sample and determining the amount of analyte recovered from the spiked sample with respect to that found in the original sample. The net measured change in concentration is compared to the expected concentration change. Influent and effluent samples were diluted into the calibration range and analyzed with and without the addition of a matrix spike. For the kit immunoassay, the PCP spike level was 15 ppb; for the plate immunoassay, the PCP spike level was 240 ppb. Spiking concentrations were chosen based on the linear range of each method. The matrix spike recovery data for each immunoassay was intended to provide information about matrix interferences in the samples after their dilution to the appropriate concentration range (Section 5). The acceptable recovery window of ±25 percent about the spike amount for the plate immunoassay was taken from the standard operating procedure for the method. The ±50 percent window for the kit immunoassay was considered reasonable for a screening method and was the limit chosen in the previous plate immunoassay evaluation (Van Emon and Gerlach, 1990).

- **QA audit samples**--These were QA standard solutions that contained specified PCP concentrations for analysis by kit immunoassay, plate immunoassay, and GC/MS for all locations. These audit samples were considered semiblind because the analyst knew they were audit samples and which dilution factors to use in preparing them for analysis, but did not know the expected concentration of PCP. Two audit sample formulations were used. One solution contained only PCP, noted as QAA; the other was a mixture of PCP and other phenols, noted as QAB. The QAA audit sample had a nominal concentration of 25 ppm PCP and was prepared by dilution from EMSL-Cincinnati QA reference standard EV-062-03-13 containing 4,950 ppm PCP in methanol (Personal Communication, 1991). The QAB audit sample had a target concentration of 20 ppm PCP and was prepared by dilution from EMSL-Cincinnati QA reference standard C-090-02, Acid Extractables II, in methylene chloride. It contained a mixture of phenols, including: phenol, 3-methylphenol, 4-methylphenol, 2-nitrophenol, 2,4-dimethylphenol, 2,4-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, nitrophenol, and a nominal PCP concentration of 1,950 ppm (Personal Communication, 1991). Both reference standards were originally developed to provide a 10 percent accuracy window for interlaboratory studies, with this window mostly due to method and laboratory variability.

  Analysis of the QAB mixture was intended to provide information on the selectivity of the anti-PCP antibodies. Both audit types were also intended to provide precision and accuracy performance data for all methods at all sites. The target PCP concentrations of 25 and 20 ppm for QAA and QAB samples, respectively, were chosen because this was expected to be the mid-range of the bioreactor sample concentrations.

- **Quality control performance samples**--These standards were designed to provide immediate information to analysts regarding the kit and plate immunoassay performance. For this demonstration, the QC performance sample concentration of 20 ppm PCP was known to the analyst and was prepared from the stock solution used for the QAA audit sample (see above). Besides its utility to the analyst regarding daily performance, the data

derived from this sample provided bias and precision estimates for the kit and plate immunoassays (Section 5).

- **Field blank samples**--These samples consisted of a reagent-grade laboratory water rinse of the decontaminated composite sampling apparatus.

- **Negative control samples**--For the kit and plate immunoassay methods, the sample dilution buffer was used as a negative control (NC) sample. These samples are useful in detecting false positive responses or contamination in the analysis.

- **Cross-calibration standards**--These standards were serial dilutions of 2,4-dinitrophenol-glycine (2,4-DNP-glycine), a yellow-colored compound. They were used to cross-calibrate the microwell-strip and laboratory-plate spectrometers. The 2,4-DNP-glycine has an absorption maximum near 405 nm, the wavelength used to measure the enzymatic product of the kit and plate sample wells.

QA/QC and Bioreactor Sample Analysis

A QC strip was analyzed at the beginning of every analysis day at each site by the kit immunoassay. The 8-well strip contained two field blank analyses (undiluted and 1:10 dilution), one NC sample, one QC performance sample, and four calibration standards. (NOTE: The four calibration standards were analyzed on every strip, not just the daily QC strip). For 3 days of each 6-day analysis week, both influent and effluent samples were analyzed; on the other 3 days, either an influent or an effluent was usually analyzed. Each influent and effluent sample was analyzed at several dilutions, both for range finding purposes and to determine the optimal dilution (i.e., in the mid-range of the calibration curve) for final sample analyses. Then, depending on the day, a series of duplicate, split, or matrix spike samples were analyzed on multiple strips. Once per week, the raw influent sample was analyzed at three different dilutions. On 3 days during each 6-day period, performance audit samples were analyzed at three different dilutions on a pair of strips. The QAA audit was analyzed on 2 of the 3 days, and the QAB was analyzed on the third day. A similar analysis scheme was followed for the plate immunoassay. The GC/MS analyses of the influent, effluent, and field blank samples followed the analysis scheme for the bioreactor demonstration (SAIC, 1989).

The QAPjP also specified the collection of field duplicate samples as part of the bioreactor demonstration. However, no field duplicate sample splits were provided by the sampling team.

Data Quality Objectives

To adequately assess the utility of the kit immunoassay as a field screening method, data quality objectives (DQOs) were proposed in the QAPjP as guidelines for evaluating the quality and validity of the data obtained in this study. However, even if the data did not satisfy the stated objectives, useful information regarding the limitations and applicability of this particular method for field testing could still be obtained. The DQOs established for the kit immunoassay in this demonstration are listed below.

1. For field (influent and effluent) samples, the test result should not differ from the GC/MS result by more than a factor of two (i.e., within 50 to 200 percent of the GC/MS result).

2. The maximum coefficient of variation (CV) for the QC performance samples, which are diluted and run by different operators on different days, should not exceed ±50 percent of the nominal concentration of 20 ppm (i.e., within 10 to 30 ppm PCP).

3. The QA audit sample results should be ±50 percent of the target PCP value (i.e., 12.5 to 37.5 ppm for QAA and 10 to 30 ppm for QAB samples).

If data from the kit immunoassay analyses of QA audit or QC performance samples fell outside the expected ranges or if blank samples exhibited contamination, then the kit immunoassay analysis for a particular strip was usually repeated (if time allowed). In addition, remaining volumes of each field sample were stored at 4 °C at each analysis site in case the data review process (Silverstein et al., 1991) indicated the need for reanalysis.

Ensuring QA Design Conformity

WBAS prepared detailed SOPs containing QC protocols and acceptance criteria for the kit and the plate immunoassays (Van Emon and Gerlach, in preparation). Coupled with the predemonstration data (Section 3), the SOPs formed the basis for the QC procedures developed for the demonstration. Additional checks were employed to ensure data conformity. For example, all micropipettors used in the study were checked for accuracy and precision by a standard gravimetric testing procedure prior to the start of formal sample analysis activities. Also, field data forms were designed so that kit immunoassay data would be collected in a consistent format from all analysis sites.

During the initial stages of the data collection activities, an on-site systems audit was conducted by EMSL-LV QA representatives familiar with the immunoassay technology. Auditors inspected on-site activities such as sample collection, handling, tracking, storage, and analyses. The results of the systems audit are summarized in Section 5.

DATA MANAGEMENT

Field data forms were used to document all pertinent information related to each sample analyzed by the kit immunoassay at all analysis sites (Silverstein et al., 1991). The forms documented analytical and field condition information, facilitated data tracking, and standardized the method by which the data were reported. The format allowed the information to be entered easily into a data base. Data generated from the plate immunoassay by EMSL-LV and WBAS were compiled in tabular form. SAIC provided copies of the data generated from their GC/MS analysis in tabular form, including sample number, PCP concentration, and data qualifiers (flags). The flags are those typically used in EPA Contract Laboratory Program (CLP) data reporting for Superfund site analytical measurements.

Upon receipt at EMSL-LV, all data were subjected to a QA review for consistency in reporting, reasonableness, transcription, and other data reporting errors. Suspicious data were flagged when appropriate to indicate observations made during the data verification and validation process. Definitions of the data qualifier flags used in this study are provided in Appendix C. Personnel at all analysis locations were contacted to verify or correct suspicious values.

After data review, the data were entered and stored in a Statistical Analysis System (SAS) data set divided into separate files (members), depending on the source of the data (on site, EMSL-LV, SAIC,

WBAS) and on the analytical method (kit immunoassay, plate immunoassay, GC/MS). The final data base from this demonstration has been fully documented in a data base dictionary. It is available for use in future assessment of the WBAS immunoassays or other immunoassay demonstrations.

SECTION 4

METHOD RESULTS AND COMPARISONS

The method comparisons and results discussed in this section pertain specifically to the analysis of the bioreactor influent and effluent samples. Field splits of the bulk influent and effluent samples were analyzed on site and off site by the kit immunoassay, off site by the plate immunoassay, and off site by GC/MS (Figure 6). Comparisons of (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 are of special interest and are discussed in detail below. Other method-to-method relationships are also addressed briefly. Results and comparisons related to various QA and QC samples are presented in Section 5.

Before the data analysis began, formal criteria for identifying a typical field analysis result had to be developed for the kit immunoassay. The protocols for this demonstration required repeated analyses of each sample at a given site. Multiple dilution levels were run to determine the optimal dilution level and replicate analyses at a given dilution level were performed to determine precision. In addition, the protocols dictated that the same samples were analyzed from duplicate independent dilutions, used in a matrix spike run, or reanalyzed at the discretion of the operator. Though results of all analyses were tabulated in the data base, only one or a pair (duplicate) of results could be used for comparison to the plate immunoassay or GC/MS results if one were to simulate a typical field analysis. Appendix D contains the algorithms that were used to represent the data values of each sample. By following these algorithms, an unbiased selection of results from the kit immunoassay analysis protocols was intended. Hence, the kit immunoassay results selected for a given sample were not necessarily the best in terms of the comparison. The sole purpose of the algorithms was to reject results if they would naturally be rejected in the field and accept the first possible results with no associated discrepancies.

Bioreactor influent and effluent samples generally represent different PCP concentration ranges; therefore, each sample type is discussed separately. Figures 7 and 8 present the results for all analysis methods at each analysis site for influent and effluent samples, respectively. Figure 7 depicts PCP concentrations for conditioned influent and raw influent samples. Conditioned influent samples were collected six times per week; raw influent samples were collected once per week. Each of the 3 weeks of sampling, represented as "A", "B", and "C", produced different ranges of concentrations for the conditioned influent samples. Conditioned influent samples collected during the first week had the lowest PCP concentration range. Sample concentrations and ranges increased during each subsequent week. This stepwise increase in concentration is related to the increased flow rate of ground water through the bioreactor, which was increased from 1 gpm during week A to 3 gpm during week B, and 5 gpm during week C. This marked difference in conditioned influent concentrations between flow rates could be caused by a "backmixing" action in the bioreactor at the lower flow rates (Stinson et al., 1991, and Appendix A). Concentration levels lower than 40 to 50 ppm were not expected for conditioned influent samples. However, when found, they proved to be a useful concentration range

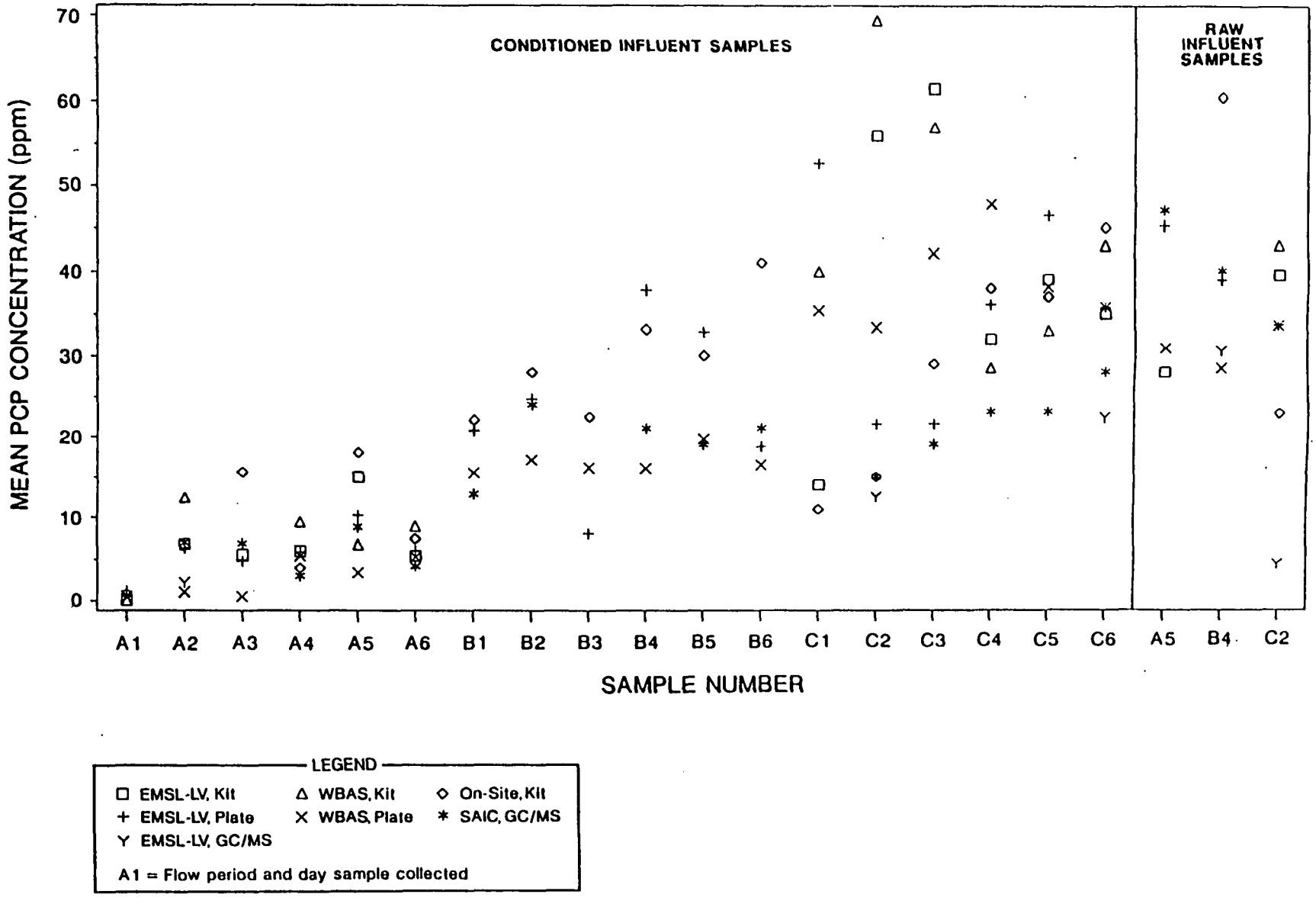

Figure 7. Pentachlorophenol concentrations for all influent samples by all methods and analysis sites over time.

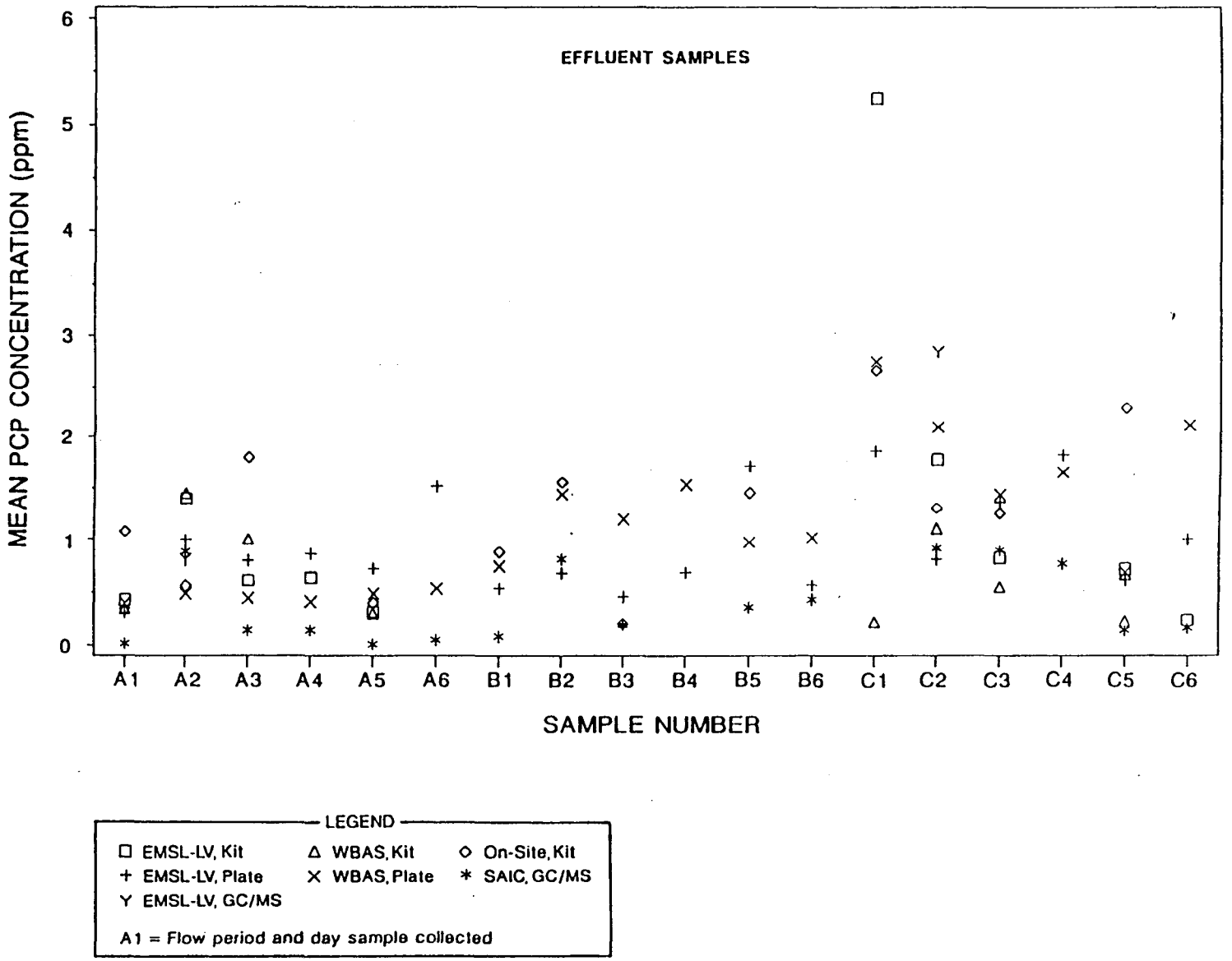

Figure 8. Pentachlorophenol concentrations for all effluent samples by all methods and analysis sites over time.

for the immunoassay demonstration. In fact, because of the quick turnaround for the immunoassay results, RREL investigators used the immunoassay data to confirm GC/MS results. This fact underscores the utility of immunoassay for on-site analysis and the advantages of conducting joint demonstrations of treatment and monitoring technologies.

Figure 8 shows that the effluent sample PCP concentration levels stayed within a relatively constant, low concentration range during the demonstration, regardless of flow rate. The ranges were slightly different for each analytical method but easily distinguishable from the higher concentration range of the influent samples. All effluent samples analyzed by all sites and methods fell below 3 ppm PCP except one outlier result (see following subsection, "On-site Kit Immunoassay Comparison to Off-site Kit Immunoassay").

Table 2 summarizes the comparison data for the most important intra- and intermethod comparisons. In Table 2, each comparison was made by arbitrarily assigning the data from one analysis site and method as Data Set 1 and the data from the comparison site and method as Data Set 2. One of the DQOs for this demonstration stated that the kit immunoassay results should not differ from the GC/MS results by more than a factor of two. As Table 2 shows, the factor-of-two DQO for the kit immunoassay influent sample results was met for only 50, 75, and 88 percent of the samples at WBAS, EMSL-LV, and on site, respectively. However, this factor-of-two comparison was used for influent samples only. Instead of using the factor-of-two comparison criterion for the effluent samples, it was more practical to compare the concentration ranges (lowest to highest, in ppm) for each method. There was no meaningful correlation between the results in the usual statistical sense for effluent sample comparisons. If one method or site reported a low PCP concentration, the other method or site also generally reported a low value within its corresponding range for effluent samples. This result is consistent with the semiquantitative nature of the kit immunoassay. Key analytical method comparisons are discussed below.

KIT IMMUNOASSAY COMPARISONS

In this section, the results obtained using the kit immunoassay are compared to the GC/MS results and the plate immunoassay results. In addition, on-site kit immunoassay results are compared to off-site kit immunoassay results.

Kit Immunoassay Comparison to the GC/MS

Kit immunoassay results were generated on site at the MacGillis & Gibbs Superfund Site and off site at the EMSL-LV and WBAS laboratories. The on-site kit immunoassay results compare favorably to the GC/MS results (Figure 9 and Table 2). A Spearman rank correlation of 0.93 ($n = 16$, 95 percent confidence interval of 0.81 to 0.98) was calculated for the influent data. This correlation indicates good relative (rank order) agreement between the kit immunoassay and the GC/MS results. Fourteen of the 16 on-site influent samples (88 percent) were within the factor-of-two objectives. Of the two samples that fell outside the limit, one is a sample for which both methods detected less than 1 ppm PCP. This sample and all effluent samples at lower concentrations are presented in an inset in Figure 9. The inset shows that the two methods provided results that were in the same general range (0.2 to 2.3 ppm PCP for the kit immunoassay versus 0.008 to 0.9 ppm PCP for the GC/MS) at these low concentration levels. (Note: other figures in this section do not provide insets as in Figure 9, but exhibit similar behavior for the lower concentration samples.)

TABLE 2. ANALYSIS SITE AND METHOD COMPARISON SUMMARY

Analysis Site and Method		Influent[a] comparison			Effluent range comparison		
Data Set 1	Data Set 2	n[b]	n<•2[c]	<•2 (%)	Data Set 1 (ppm PCP)	Data Set 2 (ppm PCP)	n
GC/MS[d]	On-site, Kit	16	14	88	0.008-0.91	0.20-2.27	11
GC/MS[d]	EMSL-LV, Kit	12	9	75	0.008-0.91	0.24-1.78	9
GC/MS[d]	WBAS, Kit	10	5	50	0.008-0.91	0.23-1.45	7
On-site, Kit	EMSL-LV, Kit	12	8	67	0.40-2.65	0.32-5.25	8
On-site, Kit	WBAS, Kit	11	6	54	0.40-2.65	0.22-1.45	8
GC/MS[d]	EMSL-LV, Plate	18	17	94	0.008-0.91	0.31-1.82	16
GC/MS[d]	WBAS, Plate	18	12	67	0.008-0.91	0.40-2.11	16
EMSL-LV, Plate	WBAS, Plate	21	16	76	0.32-1.86	0.40-2.74	18
EMSL-LV, Plate	EMSL-LV, Kit	14	10	71	0.31-1.86	0.24-5.25	10
EMSL-LV, Plate	On-site, Kit	19	15	79	0.31-1.86	0.20-2.65	12
WBAS, Plate	WBAS, Kit	12	8	67	0.40-2.74	0.22-1.45	8
WBAS, Plate	On-site, Kit	19	12	63	0.40-2.74	0.20-2.65	12

[a]Includes conditioned influent and raw influent samples.
[b]n = number of samples compared.
[c]<•2 = samples with pentachlorophenol concentrations within a factor of two (50 to 200 percent) of each other.
[d]GC/MS analyses from SAIC laboratory only.

As Figure 9 illustrates, the kit immunoassay results are systematically biased high compared to the GC/MS results. Table 3 summarizes information on high bias in the kit results relative to the GC/MS results. Due to the difference in size of the bias relative to the actual ppm values, the average bias for influent samples is given as a percentage of the values. The average bias from each analysis site for the kit immunoassay relative to the GC/MS results ranges from 65 percent to 119 percent high. Though the percent bias is larger for the effluent samples, the average actual concentration differences are not large. Hence, the average bias for the effluent samples is given as an actual concentration difference. The bias is also evident in the range of values obtained in the effluent samples. Though the net effect of the bias is marginal in terms of utility, it does minimize the potential for false negative responses from the kit immunoassay, even for low concentration samples. A similar bias is not seen when the kit immunoassay is compared to the plate immunoassay (see discussion in the next subsection).

A possible source for this bias is the cross-reactivity of the anti-PCP antibodies to other compounds in the sample. Since tetrachlorophenol had the greatest cross-reactivity, both penta- and tetrachlorophenol were quantitated by GC/MS in 8 field samples selected to span the PCP concentration range predicted from immunoassay analysis. Based on the levels of these compounds as determined by GC/MS and the cross-reactivities from Table 1, it was predicted that the kit immunoassay test results would be increased by 3 to 11 percent over the concentration expected on the basis of pentachlorophenol alone. This is much less than the 65 to 119 percent bias found between GC/MS and kit immunoassay results, indicating that other important sources of bias must be present.

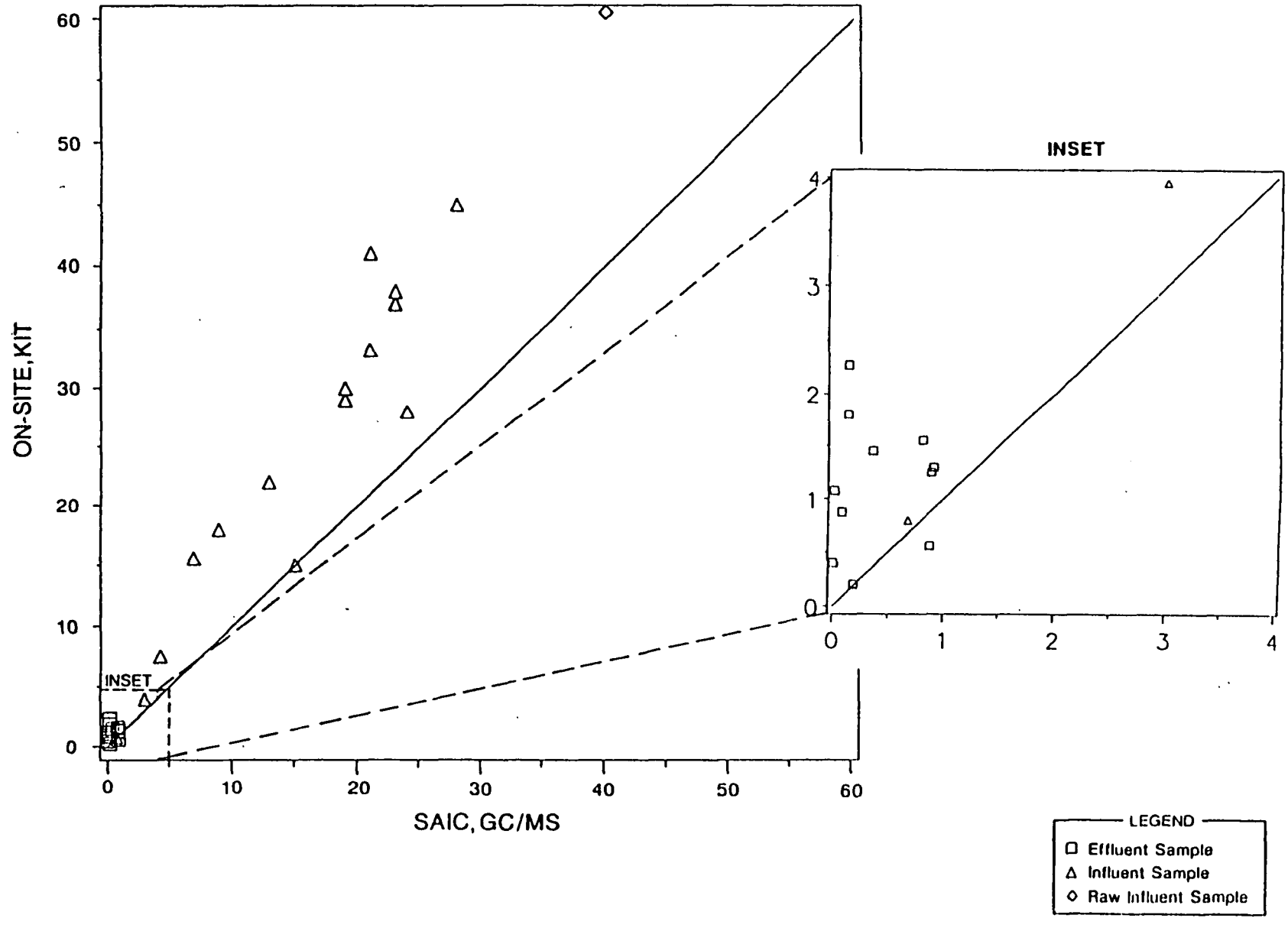

Figure 9. Comparison of results from bioreactor sample analyzed for PCP on-site by the kit immunoassay and at SAIC by GC/MS. (Units are in ppm PCP and represent mean concentrations from each analysis site.)

TABLE 3. KIT AND PLATE IMMUNOASSAY BIAS VERSUS GC/MS RESULTS

Analysis method	Analysis site	Average bias to GC/MS			
		Influent		Effluent	
		n	% Greater	n	ppm Greater
Kit immunoassay	On-site	15	65	11	0.62
	EMSL-LV	10	81	9	0.43
	WBAS	9	119	8	0.30
Plate immunoassay	EMSL-LV	16	40	16	0.55
	WBAS	16	17	16	0.55

For influent samples, the EMSL-LV kit immunoassay results compared less favorably to GC/MS (75 percent within a factor of 2; Table 2) than the on-site kit immunoassay analyses did (88 percent within a factor of 2), but trends for bias were similar (Table 3). The WBAS kit immunoassay analyses compared poorly to the GC/MS results (only 50 percent within a factor of 2 for influent samples). This result is associated with generally poor analysis results from the WBAS facility during the third week of sample analysis during which half of their analyses were performed. The laboratory problem is underscored by inconsistencies in bioreactor and QA and QC sample results generated by WBAS during this period (Section 5). These analytical problems and QA/QC inconsistencies should be kept in mind when reviewing kit immunoassay results reported by WBAS.

Of the 76 kit immunoassay analyses of the field samples, only 2 false negative results (a 2.6 percent false negative rate) were observed when compared to the GC/MS results. These two results were for the same influent sample collected at the beginning of the study, when high (50 ppm PCP) results were expected. The study design specified a minimum dilution of 1:1,000 for these samples, but subsequent analysis showed these samples to have less than 1 ppm PCP. Thus, the only false negatives reported for the field samples are associated with overdilution. It is likely that a more flexible analytical protocol would have produced a positive estimate for these samples.

Kit Immunoassay Comparison to the Plate Immunoassay

The kit immunoassay results were compared to the plate immunoassay in order to detect differences between the two methods. Figure 10 presents a comparison plot of the on-site bioreactor sample analyses for PCP by the kit immunoassay versus the EMSL-LV analyses using the plate immunoassay. This figure and Table 2 show reasonable agreement between the two immunoassay techniques (e.g., 15 of 19 [79 percent] of the influent samples analyzed on site with the kit were within a factor of two of the EMSL-LV plate results). For the kit immunoassay, 12 of 19 (63 percent) influent samples analyzed on site were within a factor of two of the WBAS plate immunoassay results. Based on plots of kit versus plate immunoassay results (Figure 10) and the ranges for effluent samples (Table 2), no significant bias was found between kit and plate immunoassay results, though they were based on different antibodies and had different cross-reactivity profiles. In addition, when the off-site kit immunoassay results were compared to the plate immunoassay results (analyzed at the same laboratory), similar variability and ranges were observed.

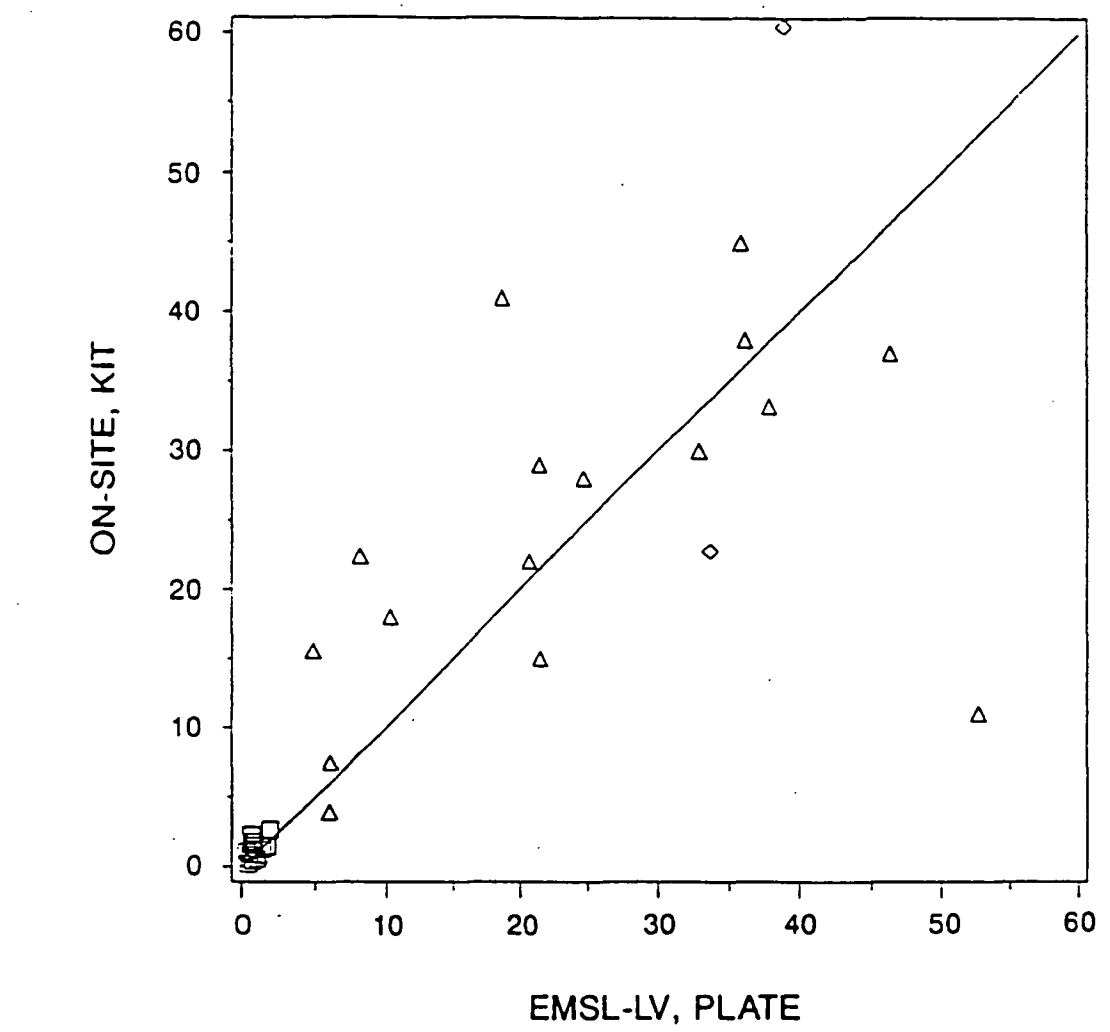

Figure 10. Comparison of results from bioreactor samples analyzed for PCP on site by the kit immunoassay and at EMSL-LV by the plate immunoassay. (Units are in ppm PCP and represent mean concentrations from each analysis site.)

On-site Kit Immunoassay Comparison to Off-Site Kit Immunoassay

The comparisons of the on-site and off-site kit immunoassay results (Table 2) exhibit the same kind of variability as is shown in the kit to plate analysis comparisons. In most cases there is similar scatter in the data with no tendency for a bias in the influent samples. Effluent samples analyzed by the kit immunoassay show ranges that are generally higher than the GC/MS and similar to the plate immunoassay. The exception to this is one unusually high effluent sample result, from a single microtiter well analyzed by the kit immunoassay at EMSL-LV, which appears to be an outlier (see Figure 8, sample C1). A misreported dilution factor is suspected in this case, though later investigation could not substantiate this possibility.

Overall Kit Immunoassay Comparison

Based on the results of the method comparisons, the kit immunoassay performed well in terms of providing a semiquantitative estimate of PCP concentrations. However, variability in the results was higher than expected based on the DQOs chosen for this demonstration. The site-to-site variability suggests significant operator-dependent or procedural contributions to the analytical error.

Some bias existed between the kit immunoassay and the GC/MS. Because the immunoassay estimate was high, a conservative estimate of PCP was made. Several factors may have contributed to this high bias. It could have been caused by the inefficiency of the extraction used in preparing a sample for GC/MS analysis. The bias could also have been related to the tendency of the immunoassay to overestimate PCP by cross-reactivity and matrix interferences.

PLATE IMMUNOASSAY COMPARISONS

In this section, the results of the plate immunoassay are compared to the GC/MS results. In addition, EMSL-LV analyses of the plate immunoassay are compared to WBAS analyses of the plate immunoassay.

Plate Immunoassay Comparison to the GC/MS

The plate immunoassay results compared more favorably to the GC/MS than did the kit immunoassay, with the exception of the results generated at WBAS for the influent sample plate immunoassay. Figure 11 presents the comparison plot of bioreactor influent and effluent samples analyzed at EMSL-LV with the plate immunoassay versus the GC/MS results. Although not as pronounced, the high bias exhibited by the kit immunoassay with respect to the GC/MS was also evident in the influent samples for the plate immunoassay (see Table 3). The plate immunoassay also shows a tendency for a higher result for the effluent sample range (0.31 to 1.82 ppm) when compared to the GC/MS (0.008 to 0.91 ppm) (see Table 2). For the EMSL-LV plate immunoassay, 17 of 18 influent samples (94 percent) were within a factor of two of the GC/MS results. WBAS reported only 12 of 18 influent samples within a factor of two of the GC/MS results. Though better than the results for the kit immunoassay, it is unexpectedly poor for the developer of the technology. Nevertheless, the data indicate that the plate immunoassay results compare more favorably to the GC/MS results. None of 78 sample analyses using the plate immunoassay generated a false negative result.

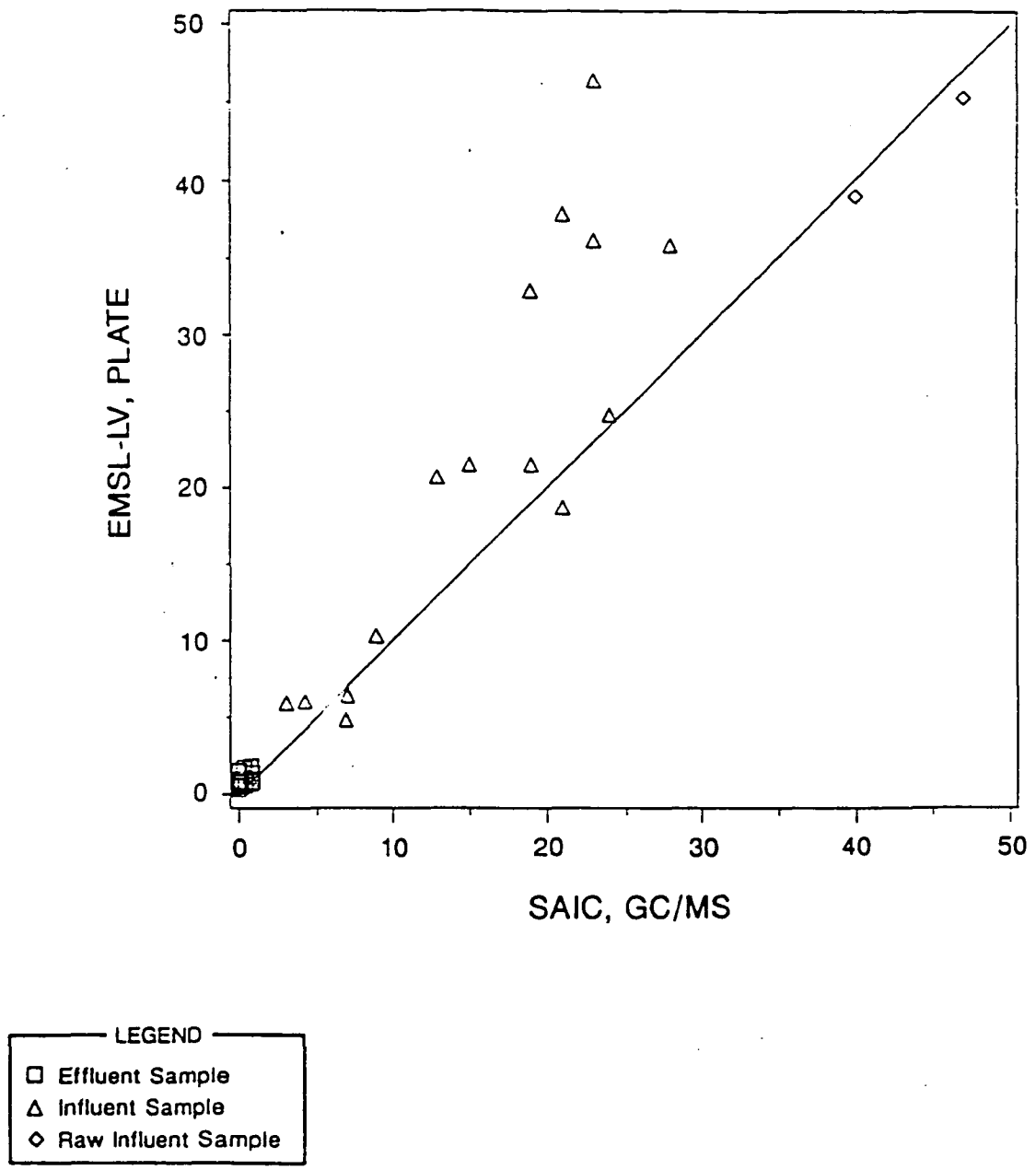

Figure 11. Comparison of results from bioreactor samples analyzed for PCP at EMSL-LV by the plate immunoassay and at SAIC by GC/MS . (Units are in ppm PCP and represent mean concentrations from each analysis site.)

Evidence that the plate immunoassay provides a closer estimate to the GC/MS than the kit immunoassay is also reflected in the high biases for the kit and plate results on influent samples (see Table 3). For an individual laboratory the average plate bias ranges from 17 percent to 40 percent too high, whereas the average kit bias ranges from 65 percent to 119 percent too high. Note, however, that for effluent samples, the bias is about the same for both immunoassay methods in terms of actual concentration (0.3 to 0.6 ppm greater than the GC/MS results).

As with the kit immunoassay, a comparison of predicted plate immunoassay to GC/MS responses was carried out for the 8 field samples based on cross-reactivity of tetrachlorophenol. The comparison predicted biases of 7 to 26 percent high for the plate immunoassay compared to the GC/MS results. The actual bias of the plate immunoassay relative to the GC/MS results was from 17 to 40 percent high. Thus, while the cross-reactivity may account for a larger portion of the bias from GC/MS results for the plate immunoassay than for the kit immunoassay, an additional source of bias is indicated.

Comparison of EMSL-LV to WBAS Plate Immunoassay Analyses

The plot of the EMSL-LV to WBAS plate immunoassay analyses of the bioreactor influent and effluent samples (Figure 12) shows poorer agreement than one would expect from a quantitative method. Sixteen of the 21 influent samples (76 percent) are within the factor-of-two objective. Even though the majority of the influent samples were within a factor of two and the effluent samples were all very close in range, there is more scatter in the data than expected between laboratories. However, the range of effluent PCP analysis results corresponded well; all 18 samples from each analysis site fell between 0.32 and 2.74 ppm PCP (see Table 2).

Although a pronounced bias was not exhibited in this comparison, a grouping phenomenon was evident in this intramethod comparison, especially in the mid-range concentrations (i.e., samples collected during the second week of the demonstration). The results from six samples analyzed at WBAS during the second week are grouped in the 15 to 20 ppm range. The EMSL-LV concentrations, on the other hand, range approximately from 10 to 40 ppm for these same samples. The grouping effect of WBAS results at the 15 to 20 ppm range by date of sample collection can also be observed at the lower and higher ranges. None of the analyses conducted at EMSL-LV showed this phenomenon. Though a definite explanation for this grouping effect could not be identified, it may be related to plate-to-plate or operator-dependent factors.

Overall Plate Immunoassay Comparison

In general, the plate immunoassay performed reasonably well in terms of its comparison to the GC/MS results and to the kit immunoassay, but the interlaboratory comparison was poorer than expected for a quantitative method. The plate immunoassay performed better in terms of accuracy and precision than the kit immunoassay (Section 5). In all cases, the effluent sample concentrations compared well between methods, and the higher concentration influent samples were generally within a factor of two of each other. Of particular note is that when the EMSL-LV plate results were compared to the GC/MS, 17 of the 18 influent samples were within the desired window; for the WBAS plate immunoassay results, 12 of 18 influent samples were within that window. The plate immunoassay, like the kit immunoassay, appeared to be biased high when compared to the GC/MS. Loss in extraction, cross-reactivity factors, or non-specific matrix effects could have contributed to this bias.

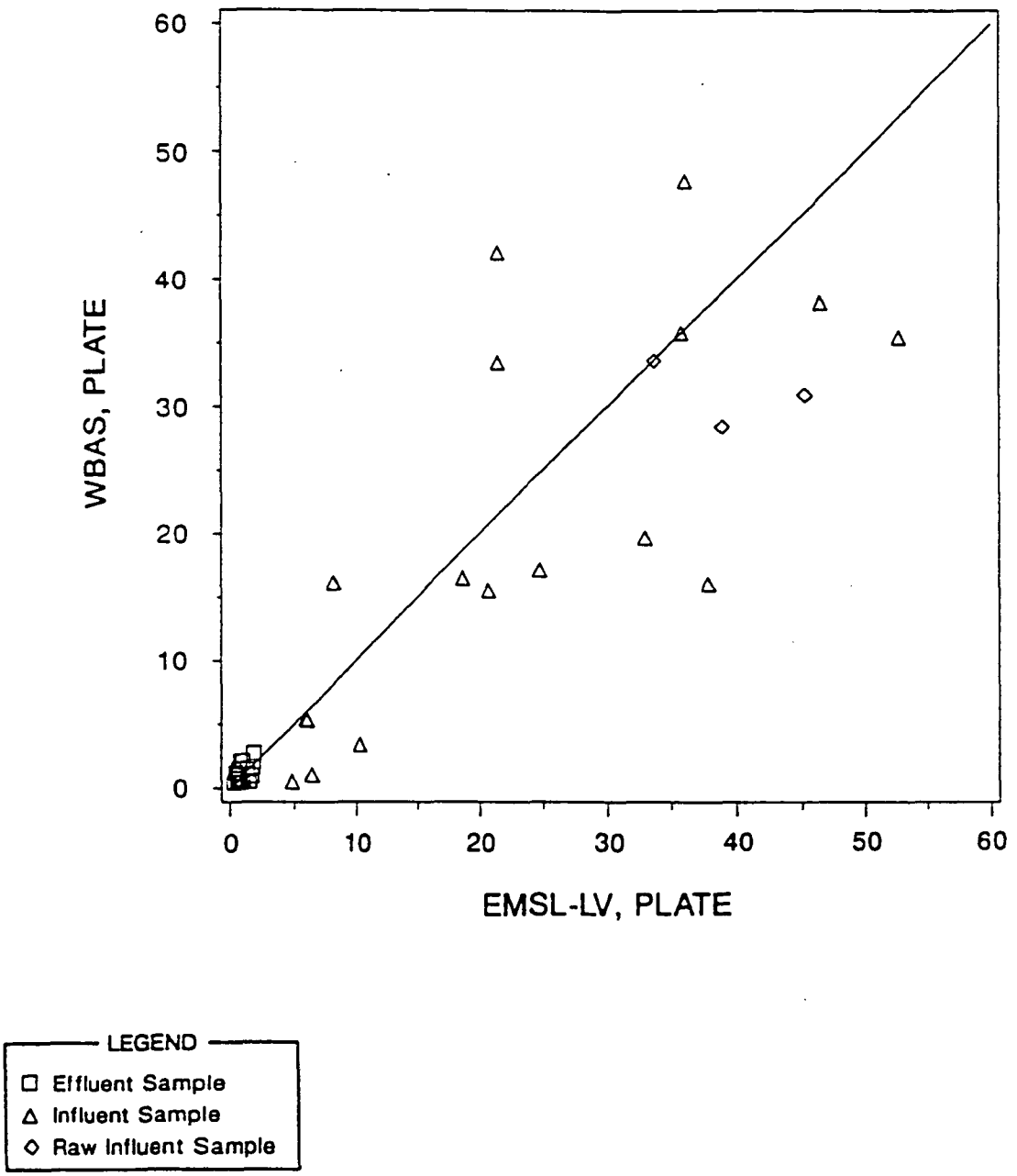

Figure 12. Comparison of results from bioreactor samples analyzed for PCP at EMSL-LV and WBAS by the plate immunoassay. (Units are in ppm PCP and represent mean concentrations from each analysis site.)

SAIC GC/MS TO EMSL-LV GC/MS COMPARISON

Though the purpose of this demonstration was not to evaluate the GC/MS method, establishing confidence in the GC/MS results was integral to the overall assessment of the utility of the immunoassay methods.

One way to assess the performance of the SAIC GC/MS data was to have an independent set of analyses of representative samples analyzed by another laboratory. To accomplish this, 1-L splits of six bioreactor samples (three influent, two effluent, and one raw influent) and one field blank sample were analyzed at EMSL-LV by the identical method (EPA Method 8270 analysis following Method 3510 extraction) (OSWER, 1986). These samples were selected at random from the complete set of samples sent to SAIC for analysis. The EMSL-LV results represent a QC check on the GC/MS results from SAIC for the entire study. Figure 13 shows the agreement between the two sets of values. The field blank is not plotted, but both laboratories reported nondetectable results. The SAIC values are approximately 30 percent higher than the values obtained by EMSL-LV. For the range of 1 to 50 ppm, the relative standard deviation of the GC/MS is 30 percent (OSWER, 1986), which means a 95 percent confidence interval of approximately 60 percent. Hence, the two laboratories agree within the accuracy limits of the GC/MS method. However, it should also be pointed out that the EMSL-LV results were obtained after the prescribed holding time for the analysis of PCP in water. As a result, effects such as analyte degradation or adsorption to sample container walls were also possibilities for the consistently lower EMSL-LV results.

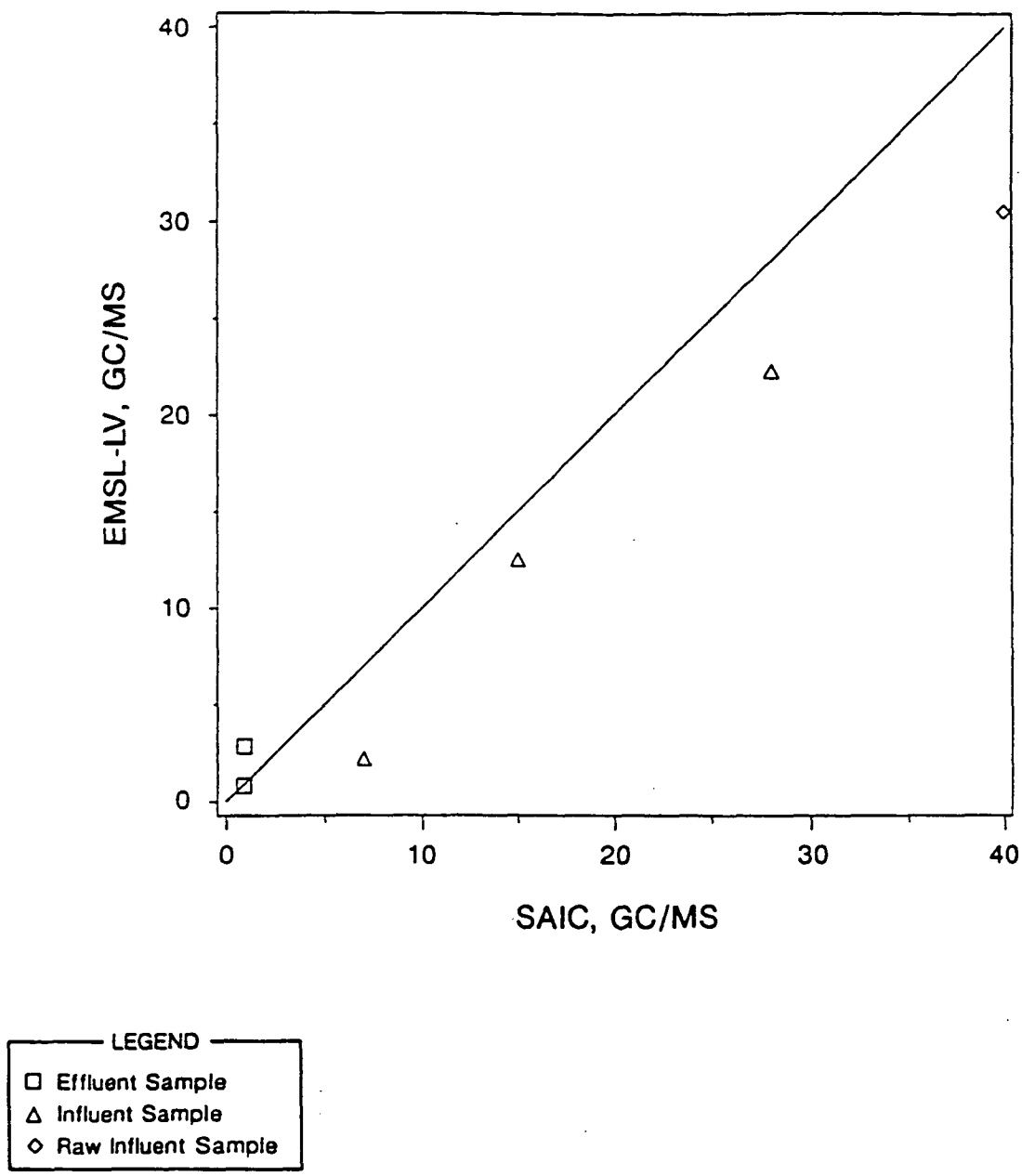

Figure 13. Comparison of results from bioreactor samples analyzed for PCP at EMSL-LV and SAIC by GC/MS. (Units are in ppm PCP and represent mean concentrations from each analysis site.)

SECTION 5

QUALITY ASSURANCE AND QUALITY CONTROL RESULTS

Though the comparisons of immunoassay technologies to the GC/MS method in Section 4 represent the most important facet of this SITE demonstration, the QA results are also important because they provide estimates for parameters such as confidence limits, detection limits, and the quality of the data. To accomplish the objectives of this SITE demonstration, the study design placed more emphasis (i.e., more QA/QC) on the kit immunoassay technology. Similarly, this section stresses the interpretation of the kit immunoassay results, but also includes information on the relative importance of plate immunoassay and GC/MS results.

A number of different types of QA and QC samples were included in the kit and plate immunoassay analyses. These included the QAA and QAB audit samples, the QC performance samples, duplicate samples, method split samples, matrix spike samples, negative control samples, and field blank samples (see Section 3 for their definitions). For comparison, only field blank samples and replicates of the QAA and QAB audit samples were run by GC/MS (Method 8270) at the EMSL-LV and SAIC laboratories. The QA and QC samples used in this study were intended to provide the following information:

- Performance characteristics (accuracy, precision, bias) of both immunoassay methods with standards of known concentration and matrix.
- Intra- and intermethod and interlaboratory comparisons.
- False negative and false positive rates.
- Data trends and correctable problems associated with the demonstration.

Acceptance criteria were established by the developer and used for screening raw data and monitoring reagent integrity. In addition, field samples were assayed with and without matrix spike samples to provide information about matrix effects and interferences. A set of serially diluted colorimetric solutions were used to cross-calibrate the laboratory and field-portable spectrometers at all applicable analysis sites.

QUALITY ASSURANCE AND QUALITY CONTROL SAMPLE RESULTS

The QA/QC sample types discussed in this section include: QAA and QAB audit samples, QC performance samples, duplicate and method split samples, negative control (NC) samples, field blank samples, and matrix spike samples.

QA Audit Samples

Two different QA standard audit samples, QAA and QAB audit samples (see Section 3), were analyzed at specified intervals at each site by each method. The QAA audit sample was prepared with a nominal concentration of 25 ppm PCP, and the QAB audit sample was prepared with a nominal concentration of 20 ppm PCP. QAA and QAB audit sample results are presented in Table 4 and Table 5, respectively.

The QAA audit sample was semiblind; analysts knew it was an audit sample but did not know the expected concentration of PCP (nominal concentration of 25 ppm). Table 4 summarizes the kit immunoassay results for the QAA audit samples in terms of the lowest dilution (1:1,000) and the mean of all dilutions (1:1,000, 1:2,000, and 1:4,000). Figure 14 provides a visual representation of this information. It is apparent that using all dilutions provides better precision only for the on-site QAA results. On-site results are both above and below the nominal concentration. Results from the EMSL-LV and WBAS laboratories indicate a systematic bias (Figure 14) above the nominal concentration. The last several QAA samples from WBAS reflect a trend that may be associated with other problems of the WBAS laboratory during the final stages of this study. The 1:4,000 diluted samples often gave the highest calculated concentrations, a problem that indicates a possible systematic error associated with dilution level. This effect could also be associated with a systematic bias at the lowest concentration level of the calibration curve. The QAA and QAB audit samples were each assayed at three dilutions, and a significant systematic error was observed only for the QAA samples.

One of the DQOs for this demonstration was that the kit immunoassay results should not differ from the nominal results for the QA samples by more than ±50 percent. Considering the QAA (1:1,000) and the QAB (mean) results for the kit immunoassay method, 25 of 34 (74 percent) were within the ±50 percent window around the nominal values. Thus, 26 percent of the QA sample values were not estimated very well with respect to the DQO. Overall, a higher percentage (90 percent) of QAB (mean) sample results were in the ±50 percent window than QAA (mean) sample results (63 percent). For both the QAA (1:1,000) and QAB (mean) results, the on-site location had the best mean accuracy, but the most variability (Figure 14). For the QAA (1:1,000) results, the WBAS laboratory had excellent accuracy, while on average the EMSL-LV results were biased high by about 40 percent (see Section 6 for a discussion of bias). For the QAB (mean) results, on-site results were biased slightly low relative to the target concentration of 20 ppm, while both WBAS and EMSL-LV produced similar results biased slightly high (Table 5). In summary, the QA results from the kit immunoassay displayed more variability than was expected, and biases were generally high compared to the nominal value.

Only two of 112 (1.8%) audit sample analyses (1 well/analysis; each dilution considered separately) generated no detectable PCP. However, each of these two false negative results had used a dilution of 1:4,000, which would have an expected concentration of 5 ppb, near the lower limit of quantitation.

For the plate immunoassay, both the EMSL-LV and WBAS laboratories generated consistent results (16 to 24 percent CV) near the target concentration for both QAA (25 ppm) and QAB (20 ppm) samples (tables 4 and 5). The only exception was one obvious outlier QAA result (giving 70 ppm) from one of three dilution levels run for one sample at EMSL-LV. The plate immunoassay QA results suggest good behavior in terms of both accuracy and reproducibility for this method. Not one of 54 plate immunoassay analyses of the QA samples produced a false negative result.

TABLE 4. PERFORMANCE AUDIT SAMPLE RESULTS FOR QAA SAMPLES

Analysis method	Analysis site	Dilution factors	n[a]	Mean conc. PCP[b] (ppm)[c]	CV[d] (%)	Samples with mean conc. outside ±50% of nominal conc.	
						Below 12.5 ppm PCP	Above 37.5 ppm PCP
Kit immunoassay	On-site	1:1,000	11	23	39	2	1
		All dilutions[e]	11	30	27	0	3
	EMSL-LV	1:1,000	5	39	24	0	1
		All dilutions[e]	5	47	30	0	2
	WBAS	1:1,000	8	25	29	0	4
		All dilutions[e]	8	42	79	1	3
Plate immunoassay	EMSL-LV	All dilutions[f]	16	21	16	0	0
	WBAS	All dilutions	16	19	16	0	0
GC/MS	EMSL-LV	N/A[g]	4	22	19	N/A	N/A
	SAIC	N/A	3	0.088[h]	13	N/A	N/A

[a] n = number of QAA samples analyzed.
[b] PCP = pentachlorophenol.
[c] Nominal PCP concentration = 25 ppm.
[d] CV = coefficient of variation.
[e] Average of 1:1,000, 1:2,000, and 1:4,000 dilution results.
[f] One outlier (at 70 ppm) not included.
[g] N/A = criterion not applicable to analysis method.
[h] Incorrect dilution scheme used in preparing the samples for analysis yielding a 0.125 ppm target concentration.

Only a few QAA and QAB samples were analyzed by the GC/MS method (tables 4 and 5). QAA and QAB results from EMSL-LV were between 14 and 25 percent low, respectively. The discrepancy from the nominal concentrations may be due to error introduced by dilution or preparation of the standard solutions or to random error in the GC/MS results. Due to miscommunication between EMSL-LV and SAIC project management, personnel at SAIC prepared QA audit samples by diluting them over 100 times more than desired (with respect to analytical protocols) prior to analysis. The results from SAIC, therefore, are not directly comparable to those from EMSL-LV. For the dilutions actually used, the expected concentrations are 0.125 ppm for the QAA and 0.040 ppm for the QAB audit samples. Thus, the average values reported are low for each type of QA audit sample.

QC Performance Samples

The QC performance samples were run once per analysis day by kit immunoassay at all sites (Section 3). Table 6 summarizes the kit immunoassay results at all sites in terms of the mean and CV for replicate analyses. The QC performance sample results are summarized in terms of the number of test results that fell within ±50 percent of the 20 ppm PCP target value. On-site QC results were

TABLE 5. PERFORMANCE AUDIT SAMPLE RESULTS FOR QAB SAMPLES

Analysis method	Analysis site	n[a]	Mean conc. PCP[b] (ppm)[c]	CV[d] (%)	Samples with mean conc. Outside the ±50% of nominal conc.	
					Below 10 ppm	Above 30 ppm
Kit immunoassay	On-site	3	16	53	0	0
	EMSL-LV	3	26	9	0	0
	WBAS	4	28	14	0	1
Plate immunoassay	EMSL-LV	8	24	24	0	0
	WBAS	8	21	16	0	0
GC/MS	EMSL-LV	4	15	10	N/A[e]	N/A
	SAIC	2	0.016[f]	47	N/A	N/A

[a] n = number of QAB samples analyzed.
[b] PCP = pentachlorophenol.
[c] Nominal PCP concentration = 20 ppm.
[d] CV = coefficient of variation.
[e] N/A = criterion not applicable to analysis method.
[f] Incorrect dilution scheme used in preparing the samples for analysis yielding a 0.040 ppm target concentration.

within this window 67 percent of the time. This is relatively poor performance and may be due to lack of training or poor field conditions. EMSL-LV had a much better rate with 89 percent within these bounds, while WBAS had a much poorer rate of only 42 percent. It should be noted, however, that all the WBAS values that were unacceptable were consecutive runs at the end of the study, a period when problems were affecting laboratory performance at WBAS.

EMSL-LV was the only site to meet the CV DQO of no more than 50 percent, with a CV of 43 percent. On-site QC values tended to be biased low (14 ppm, compared to the target concentration of 20) and had a CV of 61 percent. The WBAS QC values were biased high on average (23 ppm) and had a CV of 57 percent.

There was a relatively high rate of false negative responses for the QC performance samples (3 of 49, or 6 percent). This high rate can be partly attributed to two false negatives at the WBAS laboratory near the end of the study, when various other samples (such as audits and NCs) were also out of control. There were no false negative QC results from the on-site kit analysis.

During the early part of the demonstration, numerous plate immunoassay false positives were observed at EMSL-LV. The EMSL-LV plate immunoassay results may have been affected by (1) stability and titer of the antibody or coating antigen supplied by WBAS, (2) lyophilization of immunologic reagents, (3) contamination, or (4) some other unexplained phenomenon. This problem, which also resulted in offset standard curves and higher than expected results for the QC performance samples, was corrected by changing the antibody dilution factor (see the subsection below, "QA Problems and Resolutions.") For the plate immunoassay method, EMSL-LV results from

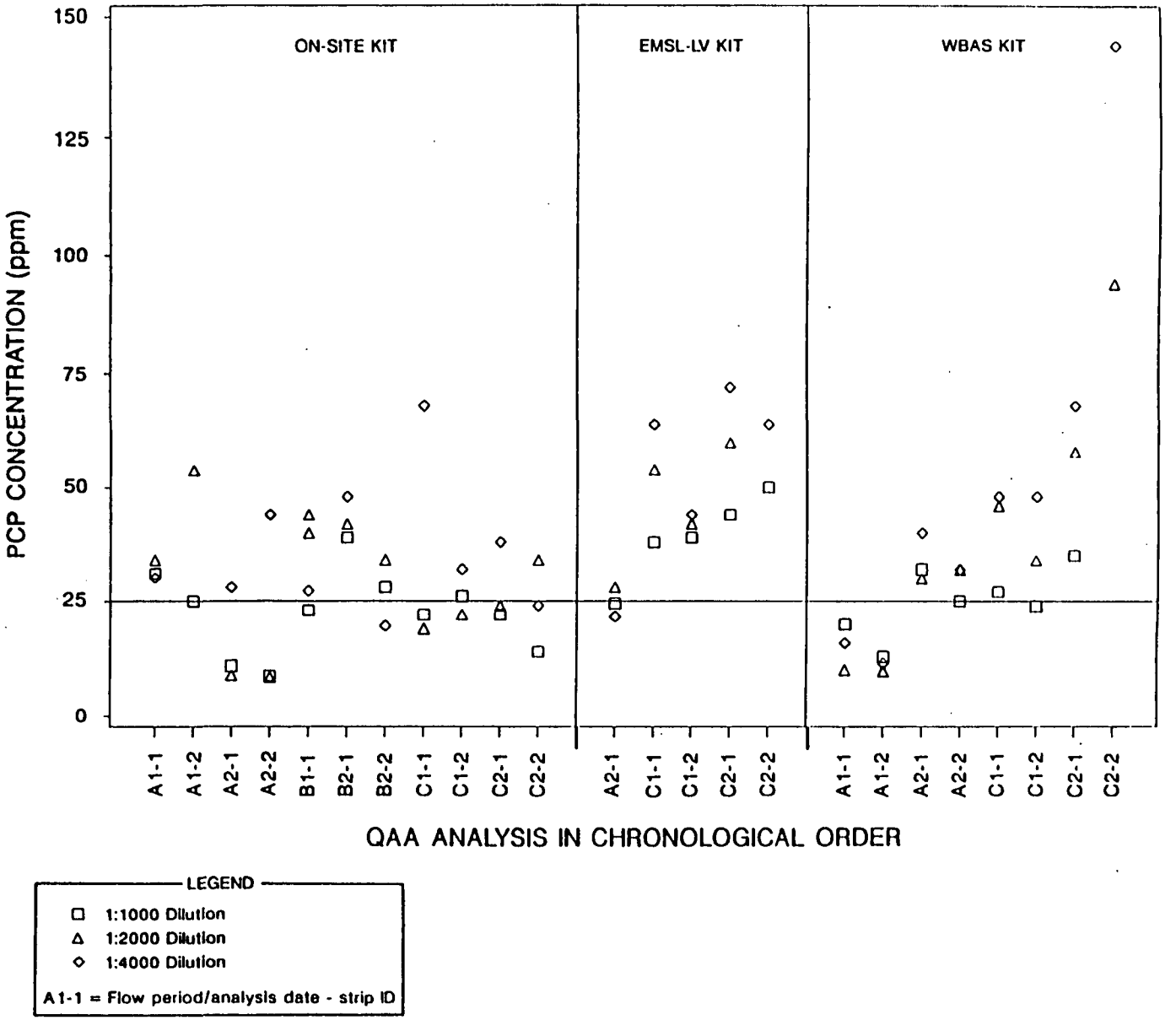

Figure 14. QAA sample concentrations (ppm PCP), determined by the kit immunoassay, by analysis site over time.

TABLE 6. KIT IMMUNOASSAY QC PERFORMANCE SAMPLE RESULTS

Analysis site	n[a]	Mean PCP[b] conc. (ppm)[c]	CV[d] (%)	Window (10 to 30 ppm)		n false negative
				n within	n outside	
On site	19 (18)[e]	14	61	12	7	0
EMSL-LV	18 (17)[e]	20	43	16	2	1
WBAS	12 (9)[e]	23	57	5	7[f]	2
All	49 (44)	19	--	33	16	3

[a] n = number of quality control performance samples analyzed.
[b] PCP = pentachlorophenol.
[c] Nominal PCP concentration = 20 ppm.
[d] CV = coefficient of variation.
[e] (n) Indicates number of sample results used in mean and CV calculation (deleted samples were either false negative or had diluted concentrations above the linear range of calibration standards).
[f] All six samples analyzed in the second half of the demonstration fell outside the window.

17 QC sample analyses generated an average 34 ppm (CV = 62 percent), and WBAS results from 12 QC sample analyses generated an average 13 ppm (CV = 8 percent). Results from WBAS were consistently low, but EMSL-LV had four results greater than 50 ppm while the rest were only slightly higher than the target value. Only three of the QC performance samples were run after the problem with the antibody was solved. The results for these analyses were within the lower portion of the performance window, with a mean PCP concentration of 14 and a standard deviation of 2 ppm. None of the 29 QC sample analyses produced a false negative result. Field samples analyzed during the period of unacceptable QC performance were reanalyzed after the reagent problem was corrected. The results from the sample reanalysis were used in the method comparison assessments (Section 4).

Duplicate and Method Split Samples

A general overview of the within-strip well-to-well variability associated with kit immunoassay analysis is shown in figures 15 and 16. Duplicate and method split samples (see Section 3 for definitions) were used in this comparison. All paired results from on site, EMSL-LV, and WBAS for both influent and effluent samples were used in these figures.

Figure 15 is a plot of the mean response for each pair versus the absolute difference in ppb units. This plot uses the "original" estimates from the standard curve for the diluted samples. Most of the differences are within 6 ppb, and this suggests that future work might require duplicate analyses (i.e., the same sample on the same strip) to be at least this close in agreement. Also, the averages of most pairs are below 25 ppb. The error appears to grow as a function of average response, which would tend to counteract dilution errors.

Figure 16 is a plot of the mean response of duplicate or split sample pairs versus their difference in ppm units. This plot represents the error after scaling up the sample concentrations by the appropriate dilution factor. The difference between most pairs is less than 50 percent of their average. This plot may be useful in establishing acceptance criteria (e.g., acceptable variability for the kit immunoassay method).

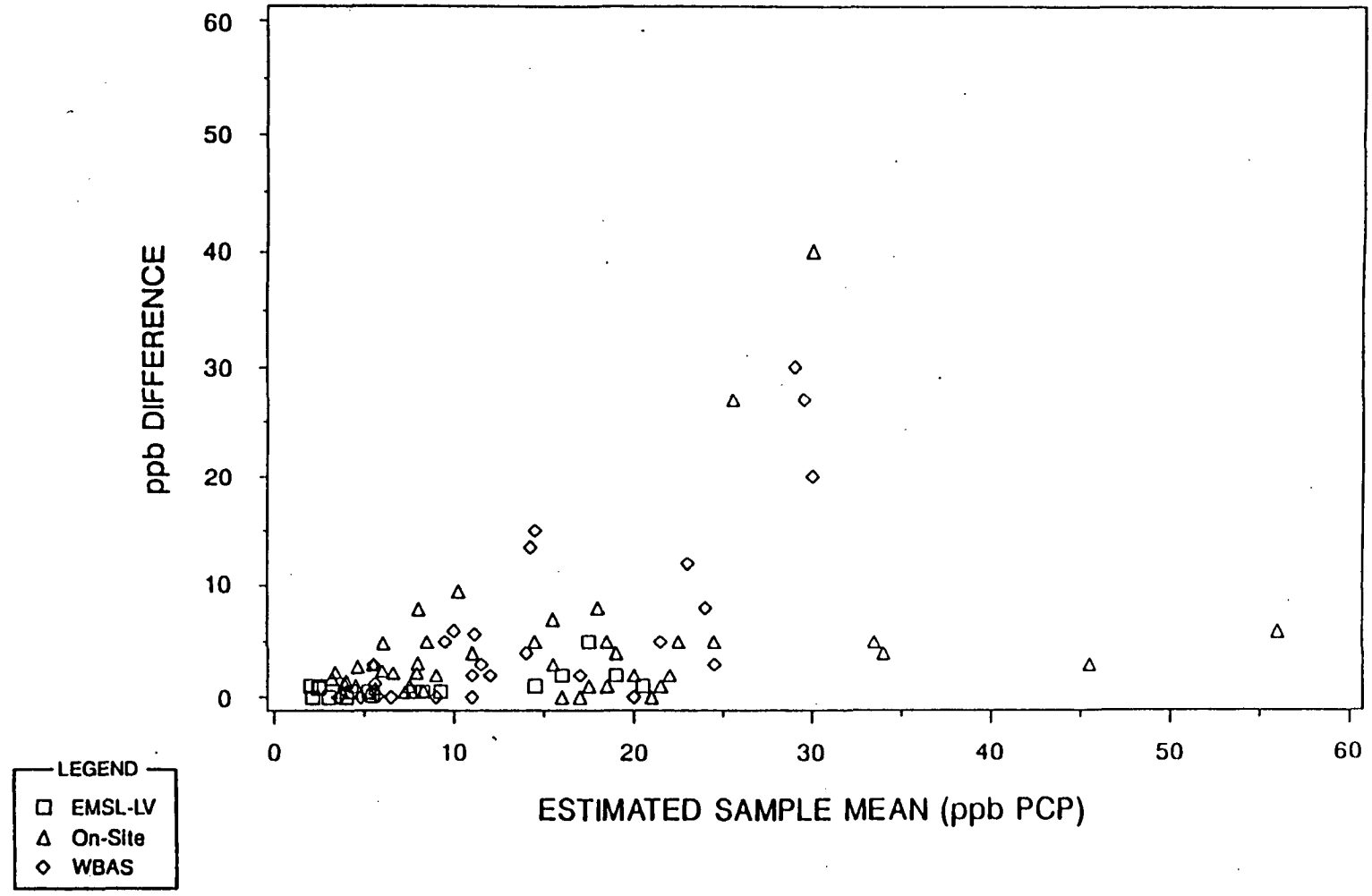

Figure 15. Mean versus difference in results from pairs of analyses for kit immunoassay duplicate and split field samples diluted into calibration range (ppb PCP).

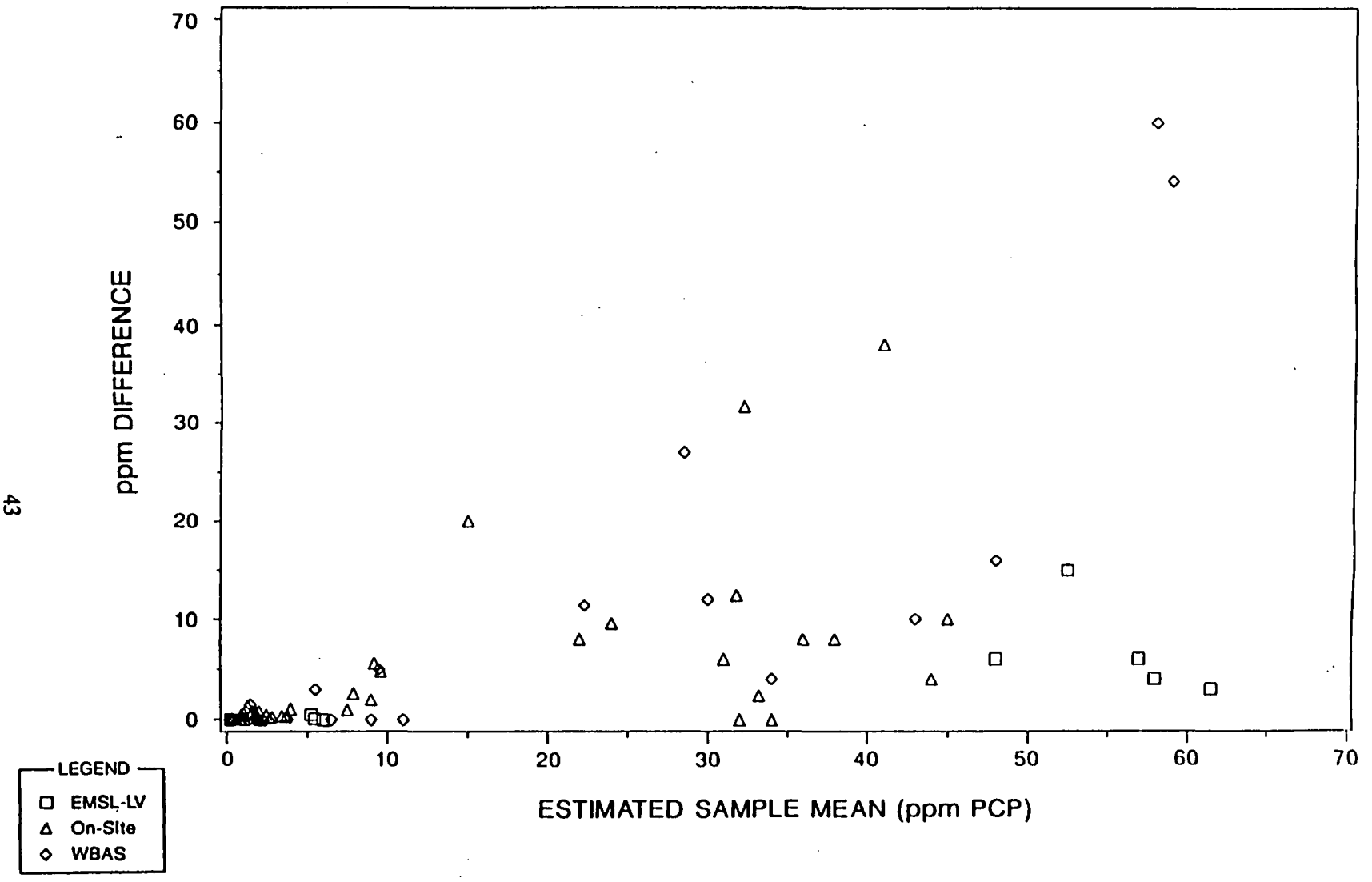

Figure 16. Mean versus difference in results from pairs of analyses for kit immunoassay duplicate and split field samples with dilution factors applied (ppm PCP).

Negative Control Samples

Negative control samples were analyzed in one well in the initial strip of each daily sample analysis series. This was one of a number of performance checks used to monitor for problems such as contamination of reagents and equipment. The overall rate of false positives for the kit immunoassay from all three laboratories was 19 percent (Table 7). The on-site rate of 22 percent was greater than the EMSL-LV rate of 13 percent. WBAS had a false positive rate of 23 percent, unexpectedly high considering WBAS was the developer. Further investigation showed that all false positives for WBAS are for samples analyzed on August 28 and 31. This time frame coincides with that of other data from WBAS suggesting poor performance characteristics during this period. The higher false positive rate seen in the on-site results is probably due to higher levels of contamination at the field laboratory and perhaps to the fact that field analysts were less familiar with the technical aspects of the immunoassay.

The overall false positive rate in the NC samples appears relatively high, but the average level is about 6 ppb. This level of contamination would not be a very significant problem if the detection limits were raised slightly. A protocol specifying a minimum value of 7 ppb would have substantially decreased the relative influence of low level contamination.

For the plate immunoassay method, none of the 9 EMSL-LV or 12 WBAS NC sample results represented a false positive. The difference between the kit and plate immunoassay results of NC false positive rates may be due to the higher detection limit (30 ppb) for the plate method.

Field Blank Samples

Daily field blank samples consisted of reagent-grade laboratory water (no detectable PCP) used as a rinse of the sample collector after it had been cleaned (SAIC, 1989). Field blanks were run once per daily sample collection series, with each immunoassay analysis performed on an undiluted sample and after a ten-fold dilution. The GC/MS field blank runs were only carried out on the undiluted sample.

The percent of positive responses for field blank samples was 13 percent for the kit immunoassay and 9 percent for the plate immunoassay (Table 8). The mean response was 5.0 ppb for the raw kit immunoassay analysis results and 34.5 ppb for the raw plate immunoassay analysis results (i.e., without the 10-fold dilution factors included). Positive field blank results were felt to represent false positive results rather than estimates of contamination of the field blanks from the sample collection decontamination rinse procedures. The pattern of false positives for the undiluted and 10-fold dilution field blank results is not statistically different (at the 90% confidence level) from what one might expect from random false positive generation. In addition, there was an absence of common false positive results for field blank samples split between sites. Hence, it is concluded that false positive field blanks are most likely due to contamination or procedural errors at the analytical level and not to contamination or carryover between samples from the sample collector. It should be noted here that the low kit immunoassay standard was 3.0 ppb, the low plate immunoassay standard was 20 ppb, and the low GC/MS standard was 20 ppb. Also, the plate immunoassay method has a lower limit of detection of about 30 ppb, and GC/MS Method 8270 has a lower limit of detection of 50 ppb. Trace levels of PCP would thus be more easily measured by the kit immunoassay than either the plate immunoassay or GC/MS.

TABLE 7. KIT IMMUNOASSAY NEGATIVE CONTROL SAMPLE RESULTS

Analysis site	n[a]	n with no PCP[b] detected	n with PCP detected[c]	Mean conc. of n where PCP detected (ppb)
On site	37	29	8 (22%)	4.6
EMSL-LV	30	26	4 (13%)	6.2
WBAS	31	24	7[d] (23%)	6.9
All	98	79	19 (19%)	5.8

[a] n = number of negative control samples analyzed.
[b] PCP = pentachlorophenol.
[c] Lowest calibration standard = 3 ppb, but detectable samples extrapolated down to 2 ppb PCP.
[d] Six of the 15 samples analyzed in the second half of the demonstration had PCP detected.

TABLE 8. FIELD BLANK ANALYSES RESULTS

Analysis method	Analysis site	Dilution factor	n[a]	n with PCP[b] not detected	n with PCP present	% n with PCP present	Mean conc. n with PCP present[c] (ppb)	Detection limit (ppb)
Kit immunoassay	On-site	Undiluted	19	17	2	--	5.6	3[e]
		1:10	20	18	2			
	EMSL-LV	Undiluted	18	15	3[d]	--	3.5	
		1:10	18	17	1			
	WBAS	Undiluted	22	18	4	--	5.6	
		1:10	12	11	1			
	All	--	109	96	13	12.7	5.0	
Plate immunoassay	EMSL-LV	Undiluted	18	18	0	--	35.4	30[e]
		1:10	18	15	3			
	WBAS	Undiluted	18	16	2	--	33.6	
		1:10	18	18	0			
	All	--	72	67	5	9.3	34.5	
GC/MS	SAIC	N/A[f]	18	17	0	--	N/A	50[g]
	EMSL-LV	N/A	1	1	0			
	All	N/A	19	18	0	0		

[a] n = numbers of field blank samples analyzed.
[b] PCP = pentachlorophenol.
[c] 1:10 dilution factor not applied in estimating the mean.
[d] One sample with a concentration above linear range of calibration.
[e] Stated detection limit (from WBAS).
[f] N/A = not applicable to analysis method.
[g] From EPA Method 8270 (OSWER, 1986).

Though there was a slightly higher rate of positive field blank responses for the kit as opposed to the plate method (13 percent versus 9 percent), analysis location, method, or dilution level did not significantly affect the percentage of field blanks giving detectable PCP. One exception was a lack of field blank samples giving positive responses for the GC/MS method; however, the GC/MS method is less sensitive. It is possible that some field blank samples were contaminated, despite the lack of statistical evidence. The collection and analysis of field blank samples entails many steps, allowing for the higher possibility of contamination for field blanks than for NC samples. However, analysis results did not corroborate this possibility. A comparison of field blank false positive rates to NC false positive rates shows that the on-site NC rate (8 of 37) is about twice as high as the field blank rate (4 of 39). For EMSL-LV, the false positive rates for the NC and field blank samples were about the same. For WBAS, the false positive rate was higher for NCs than for field blank samples.

Matrix Spike Samples

Matrix spike samples were analyzed in duplicate (i.e., two pairs per daily run) several times per week using selected influent and effluent samples. The rationale for running these samples was to obtain information about matrix effects and interferences in influent or effluent samples. Pre-spike dilution factors were selected to generate PCP concentrations in the lower part of the linear dynamic range for the kit immunoassay. Matrix spikes of 15 ppb were used at all sites, except on site for the first week when a 30 ppb spike was inadvertently used.

In general, matrix spike recoveries were highly variable for the kit immunoassay analyses. Table 9 shows that for on site and for WBAS, only 50 percent of the matrix spike recoveries were within a ±50 percent range around the expected spike recovery. EMSL-LV was somewhat better, with 75 percent within this range. The on-site recoveries were generally low but were also highly erratic. Similarly, 24 of 26 WBAS results were less than the expected result. EMSL-LV generated a slightly high spike recovery average (118 percent), but these results were also erratic.

In general, there was better performance from the matrix spiking procedure using the plate method than using the kit method (Table 10). EMSL-LV had a much wider range of percent recoveries than did WBAS, indicating that WBAS had better precision when performing the analysis of these samples. The overall percent recovery, regardless of laboratory, was slightly below 100 percent, but was still within the 75 to 125 percent window.

ASSESSMENT OF DATA QUALITY

The kit immunoassay has the potential to be used to rapidly screen samples in the field and to determine which samples should be sent to a laboratory for quantitative analysis. Consequently, the kit immunoassay must be capable of producing data which is sufficiently accurate and precise to assist the field analyst in making judgments about the source, distribution, and approximate concentration of the target analyte, PCP.

Data Quality in Terms of Five Data Quality Elements

The following sections contain a summary of data pertaining to the five basic data quality elements: accuracy, precision, representativeness, completeness, and comparability. Conclusions drawn from the data quality elements can be found in Section 6.

TABLE 9. KIT IMMUNOASSAY MATRIX SPIKE RESULTS

Analysis site	n[a]	n with recovery[b] in 50 to 150% window	n in Window (%)	Median recovery[b] (%)	Range of recoveries[b] (%)
On-site	42	21	50	67	−166 to +296
EMSL-LV	12	9	75	118	+ 57 to +313
WBAS	26	13	50	72	−146 to +186
All	80	43	54	N/A[c]	−166 to +313

[a] n = numbers of matrix spike % recoveries calculated/
[b] N/A = not applicable.
[c] % Recovery calculated as:

$$100 \frac{[\text{sample + spike}] - [\text{sample}]}{[\text{spike}]}$$

TABLE 10. PLATE IMMUNOASSAY MATRIX SPIKE RESULTS

Analysis site	n[a]	n with recovery[b] in 75 to 125% Window	n in window (%)	Mean recovery[b] (%)	Range of recoveries[b] (%)
EMSL-LV	20	10	50	87	41 to 169
WBAS	15	7	47	79	68 to 114
All	35	17	49	84	41 to 169

[a] n = number of spikes analyzed.
[b] % recovery calculated as:

$$100 \frac{[\text{sample + spike}] - [\text{sample}]}{[\text{spike}]}$$

Accuracy--

The accuracy of the plate and kit immunoassays was assessed in a number of different ways. Immunoassay results for influents, effluents, field blanks, and QA audit samples were compared directly to those obtained by GC/MS. Accuracy was also assessed by comparison of the immunoassay results with those expected for the QA and QC samples (QA audit samples, QC performance standards, NC samples). Sections 4 and 5 provide discussions of the immunoassay results on field and QA samples, respectively. Information on the preparation and composition of QA and QC samples is discussed in Section 3. Summary statements pertaining to accuracy data for the immunoassay methods are listed below.

For the kit immunoassay method:

- Seventy-four percent of all the QA audit sample analyses (n = 34) were within ±50 percent of the expected value.

- The false negative rate for replicate analysis of the nominal 20 ppm QC performance standards from all sites was 6 percent (n = 49), the rate for the influent and effluent field samples was 2.6 percent (n = 76), and the rate from the QA audit samples was 1.8 percent (n = 112). It should be noted that several instances of false negatives are associated with overdilution of the sample.

- The mean false positive rate for NC samples was 19 percent and ranged from 13 percent at one site to 23 percent at another.

- For influent samples, 74 percent of the results were within a factor of two (50 to 200 percent) of the respective GC/MS results. Percentages at individual sites ranged from 50 percent to 88 percent.

- The influent sample results were biased high by an average of 84 percent relative to the GC/MS results.

- The bias of the mean results of the QA and QC samples was site dependent. Bias ranged from near 0 to 40 percent.

- Measurable concentrations of tetrachlorophenol were found in selected bioreactor samples. The tetrachlorophenol, a cross-reactant in the kit immunoassay, could have contributed 5 to 10 percent to the high bias relative to the GC/MS analyses (Section 6).

- For all analysis sites combined, lowest dilution results were closer to the expected concentration than higher dilution results for 19 of 24 of the QAA samples and for 7 of 10 of the QAB samples.

- For the QC performance samples, 69 percent of the results were within a factor of two (50 to 200 percent) of the expected results. The percentages ranged from 42 to 89 percent, depending on analysis site.

Effluent samples with GC/MS concentrations in the range from 0.008 to 0.91 ppm PCP gave immunoassay results in the range from 0.20 to 2.27 ppm. Although the factor-of-two criterion was not always met, the data substantiate that the kit immunoassay can provide useful information about the approximate levels of PCP in environmental samples.

For the plate immunoassay method:

- Eighty-one percent of the influent sample results (n = 36) were within a factor of two (50 to 200 percent) of the GC/MS results.

- For both laboratories combined, plate immunoassay results for influent samples were biased high by 28 percent relative to the GC/MS results.

- The false negative rate was 0 percent for the bioreactor samples (n = 66), the QA samples (n = 48), and the QC performance samples (n = 29).

- The false positive rate for the NC samples was 0 percent (n = 21).

- The mean PCP result was 20.1 (n = 32), for the QAA audit samples and 22.5 (n = 16) for the QAB audit samples. These results are close to the expected concentrations for these samples (25 ppm for the QAA and 20 ppm for the QAB). The results for both sites were within ±50 percent of the expected values.

The results indicate that the plate immunoassay is quantitatively more accurate than the kit immunoassay. It is more comparable to the GC/MS results for field samples and to the expected results for the QA samples. Both the kit and plate immunoassays had low false negative rates, an essential criterion for screening methods.

For the GC/MS method, one measure of accuracy was available from the Biotrol SITE demonstration (U.S. EPA, n.d.). Eight samples were analyzed in duplicate with matrix spikes of 200 ppb PCP. Six of the eight samples had no detectable pre-spike PCP levels, and two samples had pre-spike PCP concentrations of approximately 200 ppb. The mean spike recovery was 96 percent (median recovery of 83 percent). There was considerable variability in determining percent recoveries on an individual sample basis. The range of recoveries was from 65 to 204 percent. The estimate of the standard deviation calculated from this data set was 58 percent. Based on the above, the average GC/MS recovery (based on the mean) is expected to be 0 to 10 percent low, and the typical GC/MS recovery (based on the median) is expected to be 10 to 25 percent low.

Precision--

The precision of both immunoassay methods was assessed by analyzing results obtained from replicates (duplicates and splits) of field samples, QA audit samples, and QC performance standards. Summary statements pertaining to precision data for the immunoassay methods are listed below. See sections 4 and 5 for a comprehensive discussion of the immunoassay results on field, QA, and QC samples. Information on the preparation and composition of QA/QC samples is discussed in Section 3.

For the kit immunoassay method:

- The CV for the replicates of the QC performance samples exceeded 50 percent for the combined results from all sites.

- Five out of six of the CV values for the QAA (1:1000) and QAB (mean) audit sample results were below 40 percent. The other value was 53 percent.

- The plot of mean response versus difference for field samples (Figure 15) indicated that samples analyzed in duplicate on one microtiter strip usually differed by 6 ppb or less.

- A plot (not shown) of mean response (in ppm) versus relative difference for field samples indicated that duplicates with mean concentrations above 30 ppm generally differed by 15 percent or less, while those with mean concentrations of 5 ppm or below differed by 50 percent or less.

The variability in the kit immunoassay results was higher than expected. The DQO requiring that the CV not exceed 50 percent for replicates of the QC performance samples was only met at one of the three laboratories (EMSL-LV).

The mean versus difference plots for field samples indicated that the difference between most pairs is less than 50 percent of their average and that the error appears to grow as a function of average response. The data indicate that dilution error and operator or protocol-dependent error are important factors in interlaboratory variance.

For the plate immunoassay method:

- The CV for replicates of the QA audit samples at all sites ranged from 16 to 24 percent.

- The CV for replicates of the QC performance standards was 62 percent for EMSL-LV and 8 percent for WBAS.

Overall, the plate immunoassay precision was better than the kit, which was not unexpected. Most of the CVs were in the expected range of 10 to 16 percent. The abnormally high CV for the QC performance results at EMSL-LV resulted from plate reagent performance and stability problems that were encountered during the early part of the demonstration.

For the GC/MS method, two sets of data were available from the Biotrol SITE demonstration (U.S. EPA, n.d.) from which to analyze the precision of the GC/MS results. One set of data consisted of eight field duplicate pairs. The range of CVs from these pairs was 0 to 101 percent, and the pooled CV was 33 percent.

The second set of data used to estimate precision consisted of effluent sample splits, where one split was analyzed after filtration to test whether PCP was retained on any filterable solids, such as dead cells exiting the bioreactor. Of 58 samples split for analysis, 32 splits yielded filtered results less than the unfiltered results, 22 splits yielded unfiltered results less than the filtered results, and 4 splits were identical. The ratio of higher to lower results of sample splits for testing the effects of filtration is not statistically different from 1 at the 95 percent confidence level. Therefore, the differences between pairs of results were used to generate a second estimate of precision. Since these pairs are not true duplicates, the estimate of variability derived from them will be conservative. That is, the variability estimated from them may be biased high.

The range of CV estimates for the before-and-after filtration pairs was from 0 to 115 percent. However, 80 percent of the CVs were below 27 percent, and 90 percent of the CVs were below 43 percent. The pooled CV was 21 percent, and the median CV was 13 percent. Based on both sets of precision analyses from both sets of paired samples, our overall estimate of the standard deviation for the GC/MS results is 20 to 30 percent.

Representativeness--

The data obtained for the kit and plate immunoassay methods in this demonstration are representative in two respects. Because of the extensive set of QA/QC and intermethod comparison samples analyzed in the demonstration, the results are representative of the capabilities and variability of the immunoassay methods. Also, the influent and effluent samples analyzed by the immunoassays are representative of the Biotrol bioreactor process. However, because the demonstration involved only a single site and the sample selection was limited, the data are not necessarily representative of what would be obtained at other sites or with other sample matrices. Further evaluation of the immunoassay methods may need to incorporate analysis of a wider variety of aqueous samples and matrices.

Completeness--

The completeness objective of analyzing over 90 percent of the planned number of field samples by the immunoassay methods was met (see Table 11). Another aspect of assessing completeness involved determining the number of kit immunoassay runs that gave usable results compared to the total number of runs. For the kit immunoassay, a run was defined as the results from a single 8-well microtiter strip. Table 12 gives a summary of the total number of strips run versus the number of strips yielding usable data. Approximately 7 percent of the strips run gave unusable data because no straight line could be constructed using at least 3 of the 4 standards. Thus, the 90 percent completeness objective was met for this category. For the strips with one inconsistent standard, useful data were obtained by drawing a straight line through 3 of the 4 points. Overall, 10 percent of the 256 strips providing quantifiable data fell in this category (see Table 12).

The only location that had a substantial number of strips (16) with poor calibration curves was the field site. This was probably due (in part) to a substrate contamination problem associated with the pipetting procedure. The problem was alleviated during the second week of the demonstration by a procedural modification. Out of a total of 16 unusable runs, 11 were from the first analysis week, 4 from the second week, and 1 from the third week. By percentage, this corresponds to 21 percent, 11 percent, and 3 percent of the total number of strips run in those respective weeks. These percentages substantiate the improvement in technique and performance over time.

Fewer QA audit samples were analyzed by GC/MS than the number stated in the QA plan. Due to logistical and time constraints, only 3 of 6 QAA samples were analyzed at SAIC by GC/MS, and only 4 of 6 QAA samples were analyzed at EMSL-LV by GC/MS.

Comparability--

Data pertaining to intermethod and interlaboratory comparisons are presented and discussed in sections 4 and 5. Summary statements about the immunoassay comparability data are given below.

- For influent samples, approximately 74 percent of the kit immunoassay results were within a factor of two (50 to 200 percent) of the GC/MS results. The number of results within a factor of two ranged from 50 percent at one site to 88 percent at another. The average high bias was 84 percent.

TABLE 11. TYPES AND NUMBERS OF FIELD SAMPLES ANALYZED BY ANALYSIS SITE

Sample type	Target	Kit immunoassay			Plate immunoassay		GC/MS	
		On-Site	EMSL-LV	WBAS	EMSL-LV	WBAS	SAIC	EMSL-LV[a]
Influent	18	17	12	11	18	18	16	3
Effluent	12	12	10	8	18	18	16	2
Raw influent	3[b]	3	2	1	3	3	2	2
Field blank	18	18	14	12	18	18	18	1
All	51	50	38[c]	32[c]	57	57	52	8

[a] The target values do not apply to this column.
[b] Although three samples were collected, only two were common to immunoassay and GC/MS analysis.
[c] The target value for this total is 34 because one week of analyses were not performed.

TABLE 12. SUMMARY OF QUANTIFIABLE DATA FOR THE KIT IMMUNOASSAY

Analysis site	All strips used		Strips with quantifiable data	
	n	Percent with no acceptable calibration curve	n	Percent with inconsistent standard
On-site	127	16 (13%)	111	11 (10%)
EMSL-LV	78	1 (1%)	77	11 (14%)
WBAS	69	1 (1%)	68	4 (6%)
All	274	18 (7%)	256	25 (10%)

- For influent samples, 81 percent of the plate immunoassay results were within a factor of two (50 to 200 percent) of the GC/MS results. The number of results within a factor of two ranged from 67 percent at one site to 94 percent at another. The average high bias was 28 percent.

- For influent samples, the percentage of kit immunoassay results within a factor of two (50 to 200 percent) of the laboratory plate results ranged from 63 percent to 79 percent.

- For the influent samples, both the kit and the plate immunoassay results were biased high relative to the GC/MS. On the average, kit results were biased 65 to 119 percent too high and plate results were biased 17 to 40 percent too high.

Although the percentages of kit and plate influent sample results within a factor of two of the GC/MS results were similar (i.e., 74 percent and 81 percent, respectively), the plate immunoassay results were more comparable, as indicated by the relative percentages of high bias. The broad percentage ranges for the factor-of-two comparisons (kit versus plate, kit versus GC/MS, and plate versus GC/MS) indicate higher than expected, location-dependent variability.

QA Problems and Resolutions

One of the problems encountered in analyzing the data was that the validity of the raw data from some of the strip and plate runs was questionable because of outliers, nonlinearity of the standard curve, or other problems. In some cases, different dilutions of field samples gave quite different analyte concentrations. The primary difficulty was in selecting which immunoassay data were suspect and which would be used for intermethod and interlaboratory comparisons. The QC acceptance criteria for the kit and plate immunoassay methods served as a basis for eliminating inconsistent results. Appendix D contains a discussion of the approaches taken in selecting plate and kit immunoassay data and an explanation of the rationale used in selecting data for intermethod and interlaboratory comparisons.

Another QA problem involved the GC/MS analysis of the QAA and QAB audit samples analyzed at SAIC. These ampulated samples were provided, semi-blind (with the approximate concentration ranges given), to the SAIC GC/MS laboratory in San Diego. EMSL-LV did not provide written procedures regarding sample preparation, and the concentration range given by EMSL-LV to SAIC was too broad (1 to 100 ppm PCP). As a result, the QA audit samples sent to SAIC were not diluted properly. Although unfortunate, this problem did not seriously affect project QA because: (1) the EMSL-LV GC/MS laboratory analyzed the QA samples at the proper dilution, and the measurements were within acceptable accuracy and precision limits for the method, and (2) the correlation between the EMSL-LV and SAIC GC/MS results for a number of field samples substantiated the validity of the SAIC GC/MS results (Section 4).

Difficulties were encountered in analyzing the immunoassay data because of the magnitude and complexity of the variances affecting the methods. Due to the difficulty in determining confidence intervals, it was not feasible to use overlapping confidence intervals to assess accuracy. The alternative approach was to plot the immunoassay and GC/MS results on X-Y plots and determine the scatter from the 1:1 equivalence line. As stated in the QAPjP (Silverstein et al., 1991), it was possible to determine the number of immunoassay test results within a factor of two of the GC/MS results. This was done for the influent samples that were high in PCP. However, for effluents that were lower in concentration the factor-of-two criterion was not useful. Instead, the ranges of the immunoassay and GC/MS methods were compared. The rates of false positives on the NC samples and false negatives on the QC performance standards were also used to assess accuracy. For similar reasons, the planned analysis of variance (ANOVA) could not be used to analyze sources of variance. The assumptions of the ANOVA were not met, which caused too much uncertainty to be associated with those results. Alternatively, plots of difference versus mean concentration (figures 15 and 16) were prepared for duplicates and method splits of field samples assayed by immunoassay at each location. These plots allowed easy determination of variability as a function of analyte concentration.

During early stages of the demonstration, the EMSL-LV laboratory experienced difficulty generating standard curves for the PCP plate immunoassay that were comparable to those reported by WBAS. This problem caused EMSL-LV to obtain unsatisfactory influent and effluent range-finding and QC performance sample results. After a trial and error period to isolate the reason for poor standard curve generation, the EMSL-LV laboratory obtained acceptable standard curves by adjusting the antibody concentration used in the immunoassay.

Another problem involved differences in the sampling designs of the two SITE demonstrations. Protocols for the bioreactor demonstration did not require the collection of raw influent samples. The bioreactor sampling scheme was modified to accommodate the sampling needs of the immunoassay demonstration, for which one raw influent sample was collected weekly.

Changes to the QA Plan

The following list identifies substantive changes to the QA plan:

1. The statement that all effluent samples were to be run (in the kit immunoassay) with and without a 15 ppb internal standard spike was not correct. This was in conflict with the sampling and analysis plan and should have been corrected in the original QA plan draft.

2. Contrary to the QA plan, no results for immunoassay analysis of QA audit samples were reported for the predemonstration testing phase (Section 3). The QAA and QAB samples were quantitated by GC/MS during this time.

3. A short summary report was not written at the conclusion of the preliminary evaluation phase because there was not sufficient time to analyze and interpret all the data.

Results of the On-Site Systems Audit

A questionnaire was prepared and used for the on-site systems audit that was conducted by an EMSL-LV QA representative during the first week of the demonstration. Table 13 shows the results of the audit.

The on-site auditor observed that the work area where the immunoassays were run was not kept sufficiently clean to eliminate the possibility of contamination of the highly sensitive kit immunoassay. The auditor noted the possibility of leakage from liquid waste containers and the fact that bulk sample preparation of PCP-contaminated ground water was carried out in the same area as the kit immunoassay. Though these problems were inherent to space limitations in the on-site trailer, on-site personnel should have been instructed in more detail about the precautions necessary to minimize the potential of contamination. The problems were corrected, and performance improved.

The sampling and analysis plan was followed closely, except in one instance when the raw influent sample sent to EMSL-LV and WBAS was not the same as the one that was sent to SAIC for GC/MS analysis. However, this error did not seriously affect project results and interpretations.

Sample handling, tracking, and labeling was managed well, except for one instance in which influent and effluent sample labels were switched. After a review of the results verified the problem, a correction was made by relabeling the samples in the data base.

ADDITIONAL QA/QC OBSERVATIONS AND CONCLUSIONS

Changes in Optical Density Levels of Kit Immunoassay Standards

Analysis of the OD values for the low and high (3 ppb and 40 ppb) kit immunoassay calibration standards showed two types of temporal trends associated with the standard curves. Figure 17 shows that the on-site kit immunoassay calibration standard OD levels dropped during the first week of the study and then remained generally lower than expected. Although the exact cause for this drop is unknown, it is probably associated with reagent handling, storage, and/or stability. This lowering of the OD values for the standard curve was not correlated with any change in performance. The standard curve OD values for EMSL-LV and WBAS did not show this type of trend.

The second type of change in OD readings that was seen at all three sites was a change in the difference between the OD readings for the 3 ppb and 40 ppb standards. On-site differences diminished over time, starting from a difference of about 0.4 OD on the first day and ending with differences of about 0.2 OD for the last week. An example of a standard curve generated on site during each week of the analysis is given in Appendix B. EMSL-LV differences dropped from about 0.3 OD in the first days of analysis to about 0.2 OD for samples analyzed in the third week. WBAS differences increased over the course of the first week of analysis from approximately 0.25 OD to 0.4 OD, but were generally lower in the third week (averaging about 0.2 OD, but highly variable). Whether these changes represent the effects of aging of reagents or are associated with other site-specific trends is unknown.

One of the QC acceptance criteria for raw data from the strips was that the OD for the NC samples must exceed 0.5. It was no longer possible to meet this criteria because many of the results for NC samples in the later part of the demonstration fell below 0.5. This problem was probably associated with reagent storage, handling, or stability.

Kit Immunoassay Results--Hand-Drawn Versus Computer-Calculated

All kit immunoassay results were calculated from calibration lines that were drawn by hand with a straight edge. The lines were drawn with respect to the three or four standard concentrations used. In order to determine whether this procedure was causing any systematic bias or whether noticeably improved results could be obtained using a more formal technique, the standard curves were recalculated by least-squares methods. On-site sample results were then predicted with the least-squares calibration curves. Figure 18 is a plot of the least-squares results versus the hand estimated results. This plot shows a random scatter about the line of complete agreement, with no systematic bias due to hand-drawn estimation. Plots of the least-squares results versus GC/MS results or versus plate values (not shown) were not noticeably different from those using hand-drawn calibration curves. It appears that least-squares fitting of the data would offer only marginal benefit.

Instrument Cross Calibration

Replicate analyses of the N-2,4-DNP-glycine, cross-calibration solutions on the three strip readers used in the study were evaluated in terms of relative bias and variability. In general, the mean of the three OD values obtained on different readers differed by 5 percent or less. Standard deviations for

TABLE 13. ON-SITE SYSTEMS AUDIT CHECKLIST

	Yes	Yes, with numerous exceptions	Yes, with few exceptions	No	Comment
Field forms filled out completely and accurately	X				
Kit immunoassay SOP followed	X				
QC acceptance criteria observed			X		Reagent instability used caused OD of NC to drift below 0.5
Sampling and Analysis Plan followed		X			Incorrect raw influent samples sent
Sample preparation and dilution performed as per SOP	X				One switched sample, rare 1- to 2-day lapse between collection and analysis
Sample handling, labeling, tracking and archiving performed according to instructions		X			One switched sample, rare 1- to 2-day lapse between collection and analysis
Sample packaging and shipment handled properly	X				
Safety observed		X			Except for cleanliness of work area
Cleanliness, adherence to GLP observed			X		Work area not kept clean enough to eliminate possibility of contamination

SOP = Standard Operating Procedure
QC = quality control
OD = optical density
NC = negative control
GLP = good laboratory practice

triplicates ranged from 1 to 2 percent of the means, which is within the instrument manufacturer's specifications of ±2 percent.

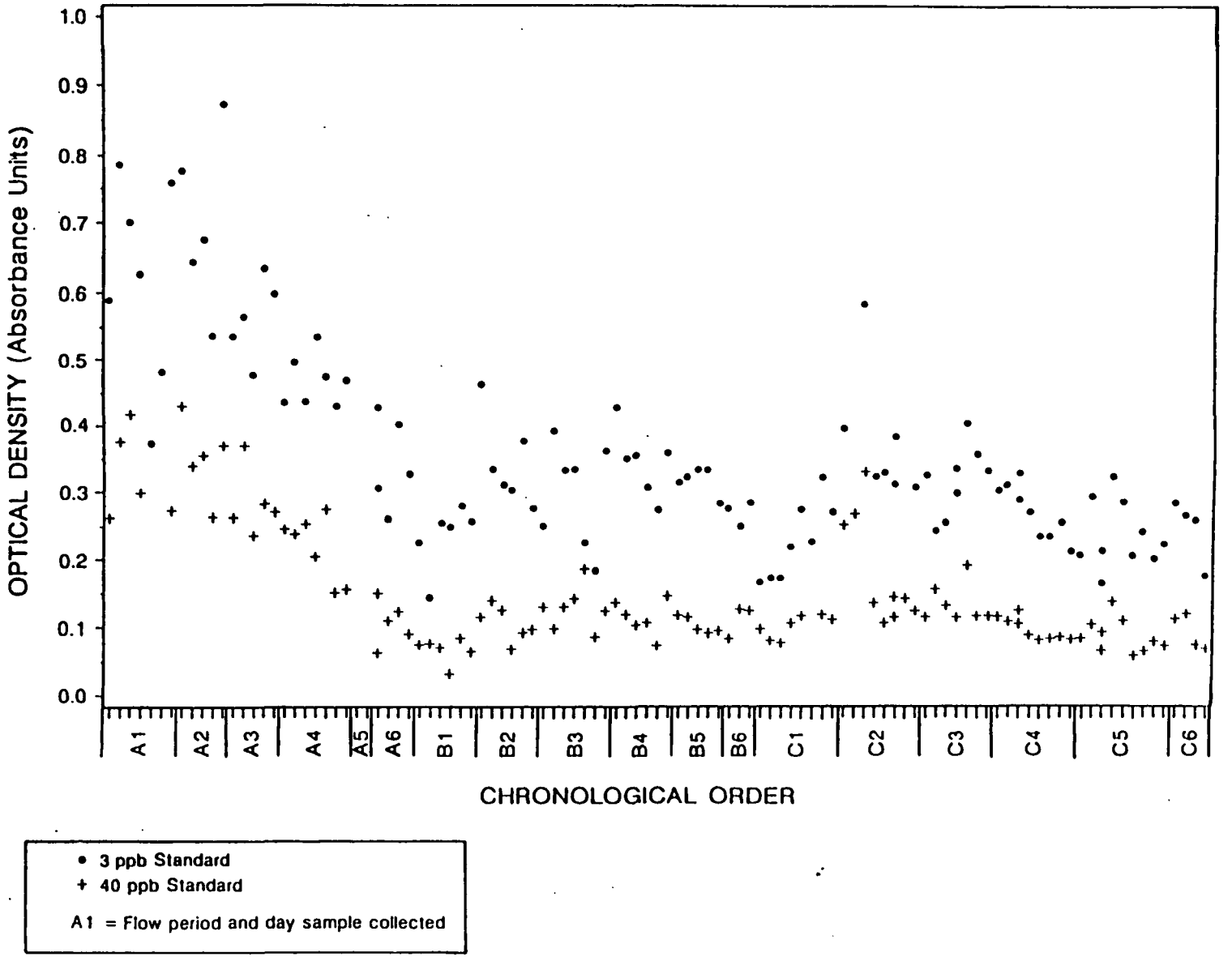

Figure 17. Optical densities of the low and high standards for the on-site kit immunoassay analyses.

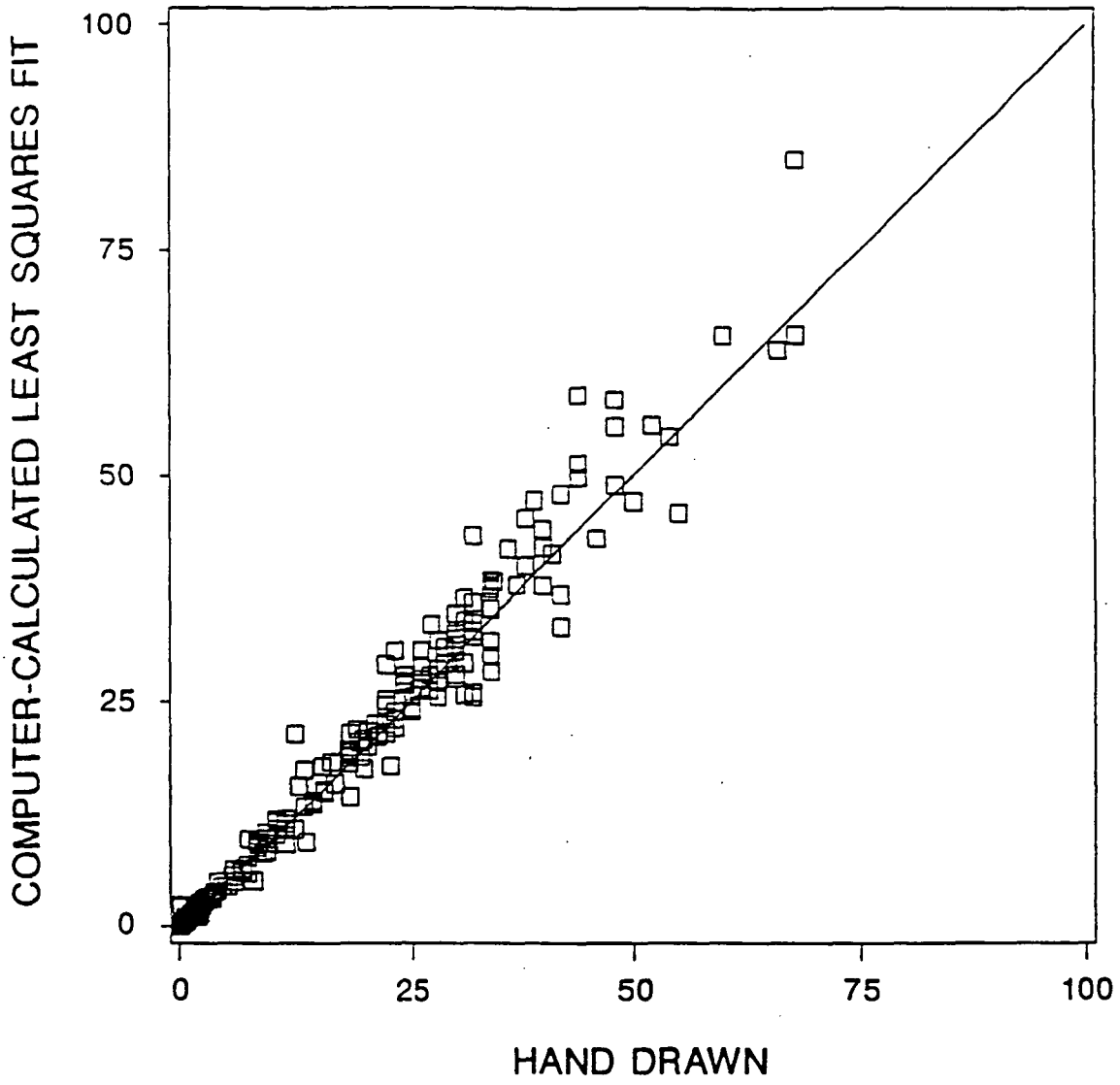

Figure 18. Comparison of the results of sample concentrations determined on-site by graph paper and straight-edge ruler plotting versus a least squares fit of the same samples. (Units are in ppm of PCP.)

SECTION 6

CONCLUSIONS AND RECOMMENDATIONS

Results of the SITE demonstration indicated that the WBAS kit and plate immunoassay technologies provide effective screening capabilities for the analysis of PCP in aqueous samples. Rapid, portable, and cost effective, the immunoassays measured approximate concentrations of target analytes, exhibited little tendency toward false negatives, and provided real-time data. The demonstration exhibited the utility of immunoassays as analytical tools that can be used in the field to complement conventional laboratory methods.

In spite of the positive results, the demonstration also reflected the need for continued development of QA/QC guidelines and protocols to improve the quality of the data. For the kit immunoassay, defining new QC acceptance criteria, raising the stated detection limit, and incorporating more procedural precautions in the SOPs should significantly improve performance. For both the kit and plate immunoassays, incorporation of stricter QC guidelines in the development of reagents could improve immunoassay reagent stability and performance.

The evaluation of the plate immunoassay was a secondary objective of this demonstration. The plate immunoassay, which was not evaluated on site, exhibited better precision and accuracy than the kit immunoassay, with quantitative results closer to those generated by the GC/MS. The plate immunoassay is field portable. Although it requires somewhat longer processing time to operate, it has a higher sample throughput than the kit immunoassay. The plate immunoassay may require more training to operate than the kit immunoassay. Like the kit immunoassay, the plate immunoassay requires additional development of QA/QC guidelines.

KIT IMMUNOASSAY CONCLUSIONS AND RECOMMENDATIONS

Kit Immunoassay Conclusions

The kit immunoassay performed well, providing a semiquantitative estimate of the approximate PCP concentrations. However, the variability of the results was higher than expected. The variability by analysis location of the accuracy and precision suggests a significant operator-dependent or procedural component in the error. In addition, data on QA and field samples run at several dilutions indicate significant systematic error associated with sample dilution.

The false negative rate was low (2 to 6 percent) and partly due to over-dilution in the sample analysis. A low false negative rate is critical for a screening method. The high false positive rate (19 percent) on NC sample analyses apparently resulted, in part, from the developer laboratory setting a method detection limit that was too low compared to the lowest detection limit of the standard curve.

The kit immunoassay results were systematically biased high compared to the results from the GC/MS analysis. A similar bias is not seen when the kit immunoassay is compared to the plate immunoassay. The GC/MS results may be biased low due to incomplete extraction efficiency during sample preparation using Method 3510 or to factors inherent to the GC/MS procedure itself. In addition, the immunoassay results may be biased high due to the reported cross-reactivity with the substantial levels of tetrachlorophenol found in the GC/MS analysis of randomly selected influent samples. However, these levels of tetrachlorophenol would not account for all the high bias based on the reported cross-reactivity (Section 5). Table 1 provides a list of cross-reactive compounds for the kit and plate immunoassays. In addition, other single-laboratory effects may contribute to the bias. The kit immunoassay replicate results on PCP standards during the preliminary evaluation phase were biased high by 30 to 47 percent. Thus, it appears that a substantial component of the bias for the kit immunoassay is inherent in the method. This error may be associated with the effects of curve fitting and linearity on method quantitation. The bias is also evident in the range of values obtained in the effluent samples. Though the net effect of the bias is marginal, it does minimize the potential for false negative responses from the kit immunoassay.

Other conclusions about the kit immunoassay are presented below:

- The factor-of-two accuracy DQO was met in most cases (88 percent for on-site analyses).

- The concentrations of the kit immunoassay and GC/MS comparison samples were in relatively close agreement. In fact, the kit immunoassay values were considered good enough to be used as a validation tool for the GC/MS results in the BioTrol bioreactor demonstration. When the GC/MS concentrations for influent samples were unexpectedly low, EMSL-LV was contacted by the RREL data interpretation and QA staff to find out if the immunoassay results were also low. This confirmation allowed RREL to investigate other factors as to why the influent samples were so low.

- The kit immunoassay was able to detect the same basic trends in the samples collected from the bioreactor as the GC/MS. These findings included the high concentrations and wide ranges of influent samples (~0.1 to 50 ppm PCP) and the relatively low and constant concentrations of PCP (~0.01 to 3 ppm) detected in the effluent samples.

- The kit immunoassay method is quicker and requires less technical skill than the GC/MS. The kit immunoassay results were obtained on site and within hours of sample collection, whereas the GC/MS requires a minimum of several days for sample shipment and off-site sample extraction and analysis steps. Field personnel were trained in 4 hours to use the kit immunoassay. However, the kit immunoassay responds only to PCP and to a lesser extent to structurally similar compounds, while GC/MS can identify and analyze a wide spectrum of organic compounds.

- The variability of the kit immunoassay was higher than desired, based on the results from QA and QC performance samples that fell within ±50 percent of the nominal concentrations. Precision ranged from 25 to 60 percent, depending on the performance (QA/QC) sample type and the analysis location. The variability observed in this study would categorize the kit immunoassay as a semiquantitative method.

In addition, the kit immunoassay method generates far less hazardous waste than the GC/MS (<10 mL aqueous wash versus 1 L of methylene chloride per sample analysis). Plotting calibration curves by hand and estimating concentrations using graph paper did not produce significantly different results from sample concentrations calculated with computer-based, least-squares methods.

Table 14 presents a comparison of method performances and other critical comparison parameters related to methods for analyzing PCP in aqueous media (modified from Van Emon and Gerlach.1990). The percentages given for accuracy and precision, for the immunoassays and EPA Method 8270, were those found in this demonstration.

Kit Immunoassay Recommendations

The kit immunoassay shows potential as a technique that can be used as a semiquantitative field screening method for site characterization and remediation activities. The method should be used in conjunction with initial confirmatory analyses to assess possible site-specific or matrix interferences. In addition, the kit immunoassay could be used to check for contamination of field blank samples and sampling equipment.

The usefulness of the kit immunoassay can increase with refinement in various procedural, documentation, and QA/QC limits and confidences. Method improvement recommendations include:

- Defining new QC acceptance criteria for raw data, such as:

  a) maximum (±) differences between duplicate samples at high and low concentrations.

  b) tests for linearity of the calibration curve.

  c) minimum OD value for NC samples.

- Raising the stated level of detection to lower the false positive rate (e.g., mean response for NCs + 2 standard deviations).

- Rewriting the kit immunoassay SOP to emphasize stricter adherence to critical procedural steps to improve on precision and accuracy of the method (e.g., strict adherence to pipetting protocols to limit substrate contamination).

- Improving QC protocols in the formulation of the immunoassay reagents by:

  a) documenting the shelf life and stability claims (e.g., temperature affects) for all kit reagents.

  b) reformulating reagents to improve stability, if necessary.

- Attempting to expand the relatively narrow linear dynamic range (3 to 40 ppb) of the calibration curve by plotting data on log-log or log-logit plots or by adjusting the levels of antibody or enzyme-labeled conjugate.

TABLE 14. COMPARISON OF METHOD PERFORMANCES FOR PCP ANALYSIS IN AQUEOUS SAMPLES

Performance parameters	WBAS, kit immunoassay	WBAS, plate immunoassay	EPA Method 8270, GC/MS[a]	EPA Method 604, GC[b]
Detection limit (ppb)	3-5	30-40	30-50	1-15
Linear dynamic range (ppb)	3-40	30-400	30-200	1-200
Precision[c]	±30-40%	±20-30%	±20-30%	±20%
Accuracy[c]	±50%	±40-50%	-10 to -25%	±30%
Analysis time based on sample load (detection only)	0.5 hour/10 samples	2.5 hours/40 samples	1 hour/1 sample	0.5 hour/1 sample
Extraction required	No	No	Yes	Yes
Cost/sample	$7.50	$2.50	$300-$750	$100-$300
Key interferents	2,3,5,6-tetra-chlorophenol	2,3,5,6-tetra-chlorophenol	Various	Polyaromatic hydrocarbons, matrix dependent
% matrix spike recovery	75-125%	75-125%	10-95%	20-80%
Rapid on-site analysis capability	Yes	Yes	No	No
Total analysis time[d]	1.5 hours/10 samples	5 hours/40 samples	5 hours/1 sample	4.5 hours/1 sample

[a] From Test Methods for Evaluating Solid Waste, SW-846, (OSWER 1986).
[b] From Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, (EPA 1982).
[c] Results from this field and earlier laboratory studies.
[d] Includes extraction, cleanup, detection, quantification, data package assembly, and associated quality assurance.

- Replacing the 8-well microtiter strip format with 12-well, microtiter strips (or 2 by 8 well strips) so that negative and positive control samples and performance samples, along with unknowns (i.e., environmental samples), can be included in each run.
- Using precision pipettors as standard equipment in the field kit.
- Investigating the cause(s) of the bias between the immunoassay and the GC/MS results.

PLATE IMMUNOASSAY CONCLUSIONS AND RECOMMENDATIONS

Plate Immunoassay Conclusions

The accuracy and precision of the plate immunoassay were generally better than for the kit immunoassay. The plate immunoassay data were also more comparable to the GC/MS data. In addition, quantitation of the QA audit samples was more accurate and precise, and the false positive and negative rates were lower. However, the initial problems with the reagents forced the reanalysis of a large quantity of samples at one analysis site.

The plate immunoassay performed reasonably well in terms of its comparison to the GC/MS results, the kit immunoassay results, and the interlaboratory results. In all cases, the effluent sample concentrations compared well across all methods, and the higher concentration influent sample results were generally within a factor of two of each other. In fact, the results for 17 of the 18 influent samples analyzed at EMSL-LV by the plate immunoassay were within a factor of two of the GC/MS results. The plate immunoassay, like the kit immunoassay, appeared to be biased high when compared to the GC/MS. The loss in extraction and cross-reactivity factors could have caused much of this high bias. However, when compared to each other, the immunoassay technologies exhibit no bias.

Summary conclusions about the plate immunoassay are presented below.

- There was good relative agreement between the plate immunoassay and the GC/MS results.
- The plate immunoassay proved to be more quantitative than the kit immunoassay; however, the plate immunoassay results were more variable than desired. This is evident particularly when the EMSL-LV and WBAS laboratory plate immunoassay results are compared.
- No false negative responses were generated by the plate immunoassay based on the influent and effluent field samples and the QA audit and QC performance samples.
- No false positive responses were generated by the plate immunoassay based on the NC samples.
- The information obtained on the performance of the plate immunoassay provided important supplementary data for the previous study, which dealt primarily with surface, drinking, and ground water spiked with PCP. The SITE demonstration data added the aspect of environmental water samples contaminated with PCP to the assessment of the plate immunoassay results presented in Van Emon and Gerlach (1990).

Plate Immunoassay Recommendations

The plate immunoassay can be useful for the analysis of PCP in water samples. Although it is field portable, the plate immunoassay is more complex to perform than the kit immunoassay. However, since it is more quantitative than the kit immunoassay and has a larger sample throughput per run (i.e., 96-well microtiter plate versus 8-well strips), the plate immunoassay presents some advantages over the kit immunoassay in both fixed laboratory and field laboratory environments.

The technology could be improved if more thorough QC protocols were developed in the formulation of the immunoassay reagents. These protocols are needed to document the shelf life and stability claims for all reagents, especially the anti-PCP antibody, and to reformulate the reagents to improve their stability and uniformity. Other conclusions and recommendations concerning the plate immunoassay can be found in Van Emon and Gerlach (1990).

JOINT SITE DEMONSTRATION CONCLUSIONS

The WBAS immunoassay demonstration reflected the advantages of joint SITE demonstrations. The bifocal nature of this SITE demonstration proved timely and cost effective. It also showed that one set of confirmatory methods can be used to assess multiple technologies if analytical controls on these methods are understood by all demonstration participants. This joint demonstration underscored the importance of careful planning, organization, coordination of effort, and communication among participants.

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APPENDIX A

SITE DEMONSTRATION OF BIOLOGICAL TREATMENT OF GROUNDWATER BY BIOTROL, INC. AT A WOOD PRESERVING SITE IN NEW BRIGHTON, MN

by

Mary K. Stinson, ORD/RREL-USEPA, Edison, NJ
William Hahn, SAIC, Paramus, NJ
Herbert S. Skovronek, SAIC, Paramus, NJ

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ABSTRACT

A wood preserving site in New Brighton, MN on EPA's National Priorities List was selected for evaluation of a groundwater treatment for pentachlorophenol with a fixed-film biological system. The system employs indigenous microorganisms but is also amended with a specific pentachlorophenol-degrading bacterium. The mobile, pilot-scale unit used for the demonstration houses a 540 gallon, three-stage bioreactor filled with structured PVC packing for biomass support. After an initial acclimation period, groundwater from a well on the site was fed to the system at 1, 3, and 5 gpm with no pretreatment other than pH adjustment, nutrient addition, and temperature control. Each flow regime was maintained for about two weeks while samples were collected for extensive analyses.

At 5 gpm, the system was capable of achieving about 96% removal of the pentachlorophenol in the incoming groundwater and producing effluent pentachlorophenol concentrations of about 1 ppm, which met the local POTW requirement for discharge. At the lower flows (1 and 3 gpm) removal was higher (about 99%) and effluent pentachlorophenol concentrations were well below 0.5 ppm.

Operating costs, including power (pumping of liquids and heating), nutrients and caustic, and operator labor, are reported. This system appears to be a compact and cost-effective treatment for pentachlorophenol-contaminated wastewaters. Pre- and post-treatment such as for oil or solids removal, may be required on a site- and wastewater-specific basis.

The results reported in this paper are preliminary and a full report is in preparation. This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.

INTRODUCTION

Soil and groundwater contamination by chemicals commonly resulting from wood preserving operations has frequently been found at Superfund sites on the National Priorities List. Under the Superfund Amendments and Reauthorization Act of 1986 (SARA), the U.S. Environmental Protection Agency was empowered to initiate a Superfund Innovative Technology Evaluation (SITE) program to develop, demonstrate, and evaluate new and innovative technologies that could be used at Superfund sites. A method for the destruction or removal of hazardous chemical species such as pentachlorophenol (PCP) and creosote-derived polynuclear aromatic hydrocarbons (PAHs) found at wood preserving sites was deemed to be suitable for investigation under this program.

BioTrol, Inc. of Chaska, MN offered a biochemical destruction technology and encouraging claims from earlier, small-scale studies that indicated that efficient removal of such pollutants from contaminated soil and groundwater could be achieved. While biotreatment has a long history as a cost-effective destructive method for organic chemicals in both industrial and municipal wastewaters, it was uncertain whether such technology would be effective at Superfund sites for the recalcitrant chemicals that might be encountered as a result of long term wood preserving operations, specifically pentachlorophenol and polynuclear aromatic hydrocarbons.

Subsequently, the BioTrol, Inc. Aqueous Treatment System (ATS) was selected for investigation under the SITE program. After considering alternate sites, a facility recently added to the National Priorities List was chosen for a pilot-scale evaluation of the technology. The selected site, in New Brighton, MN, a suburb of Minneapolis, has been used for wood treatment with various preservatives, including creosote, pentachlorophenol, and chromated copper arsenate since the 1920s. Tests at the site as part of a RI/FS indicated that both the soil and the underlying groundwater were contaminated with pentachlorophenol and polynuclear aromatic hydrocarbons, even though these chemicals were no longer being used in wood treatment. The owner and operator of the site, the MacGillis and Gibbs Company, agreed to host the pilot scale testing of the BioTrol system.

PROCESS DESCRIPTION

The BioTrol Aqueous Treatment System (ATS) shown in Figure 1 consists of a conditioning or temper tank, a heater and heat exchanger, and a three-stage fixed-film biological reactor. Incoming wastewater is first brought to the conditioning tank where the pH is adjusted (if necessary) to just above 7.0 with caustic and inorganic nitrogen and phosphorus nutrients are added. After passing through the in-line heater and heat exchanger to assure a more constant temperature in the vicinity of 70 F, the wastewater is introduced to the base of the first of the three bioreaction chambers (Figure 2). Each chamber is filled with an inert support for bacterial growth; in the study corrugated polyvinyl chloride sheets were the support medium used (Figure 3). The influent is passed up through each chamber while air is injected at the base of each chamber through a sparger tube system, as shown in Figure 2.

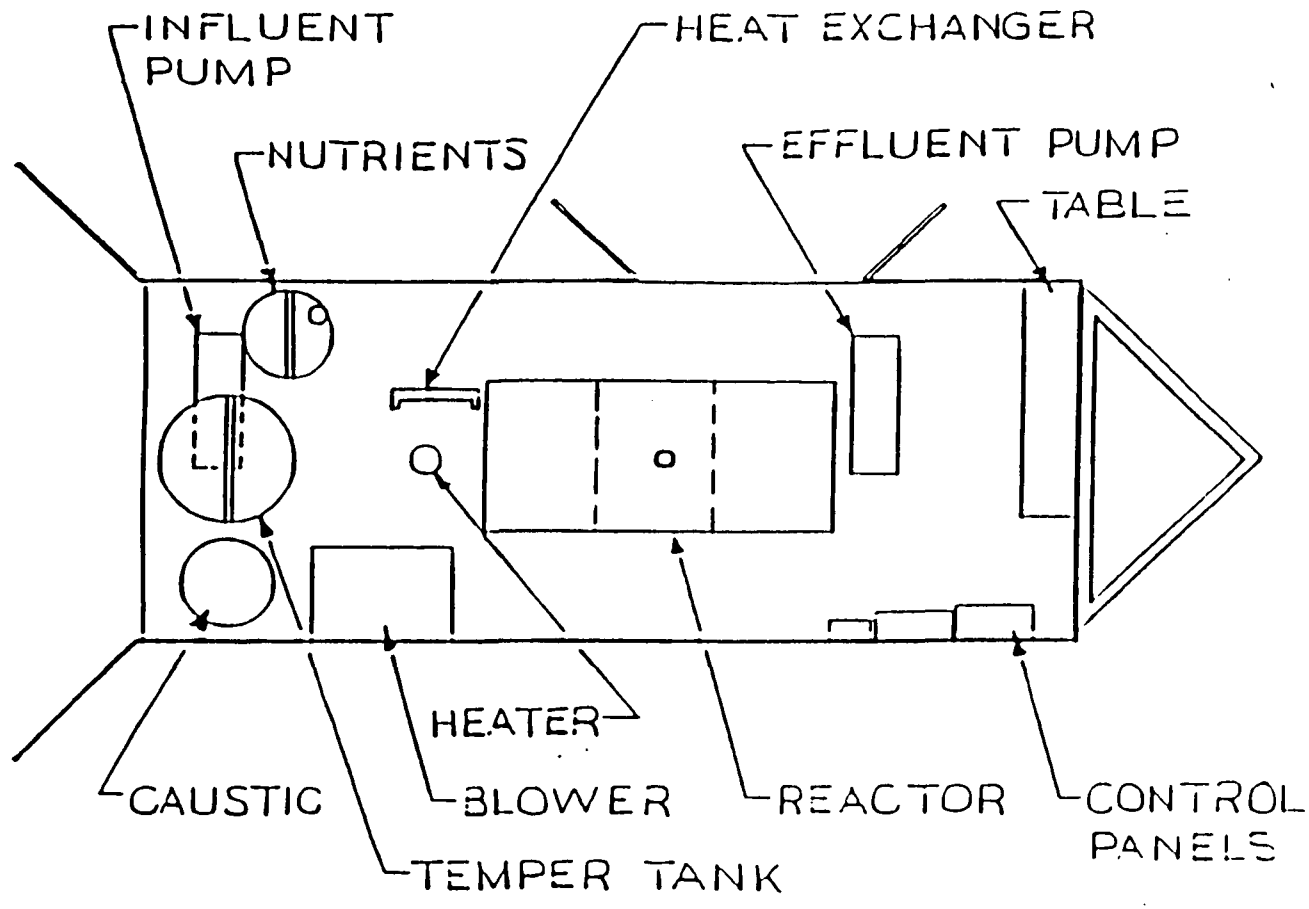
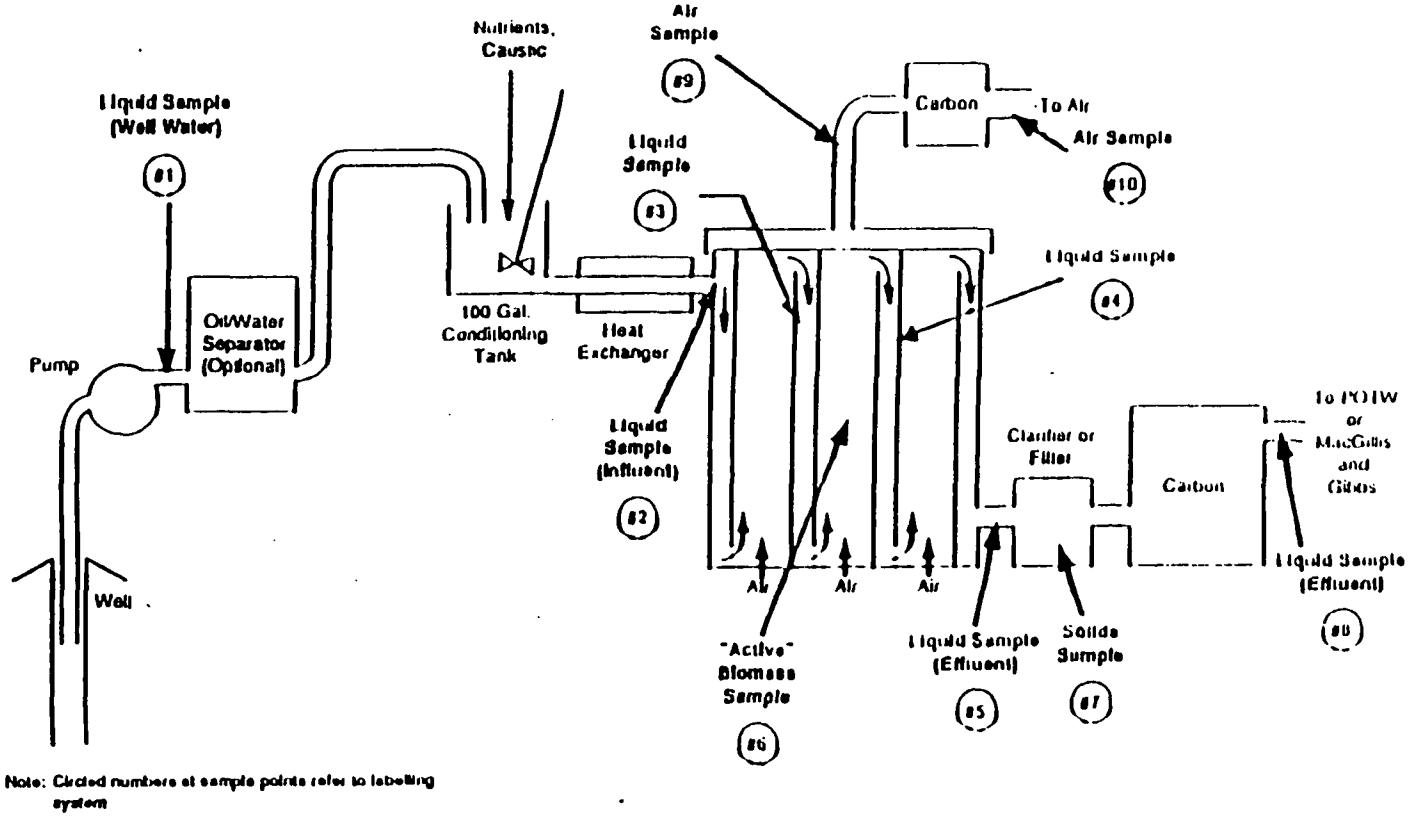


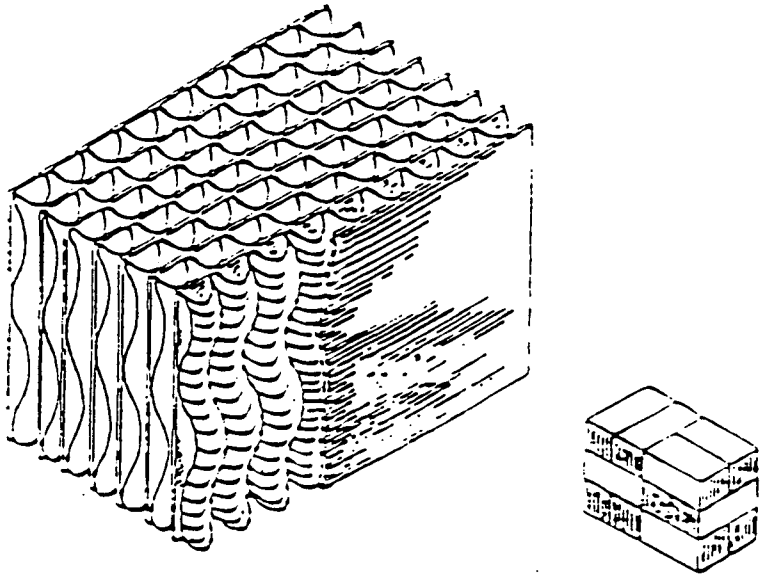
Figure 1. BioTrol, Inc. Mobile Aqueous Treatment System (ATS) System.



Note: Circled numbers at sample points refer to labelling system

Figure 2. Aqueous Treatment System (ATS) with Sampling Points Shown.

FIGURE 3.
CORRUGATED POLYVINYL CHLORIDE MEDIA



The system is acclimated by introducing an indigenous bacterial population taken from the local soil. After allowing about one week for acclimation of this growth to the wastewater, the system is "seeded" with an inoculum of a Flavobacterium specific to the target contaminant, in this case pentachloropnenol. The wastewater containing the contaminant is then recycled through the system to allow the bacterial population to readjust. When the system is fully adapted to the wastewater, once-through processing is ready to begin.

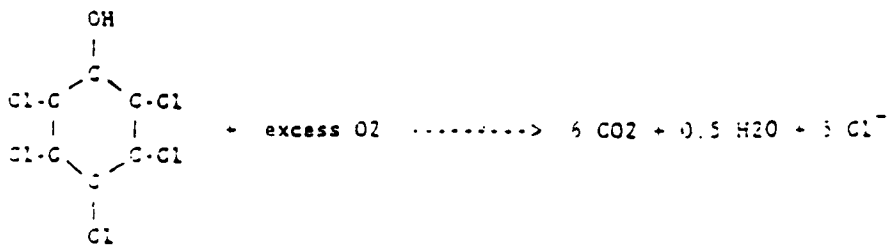
At the MacGillis and Gibbs site it was determined that the quality of the groundwater did not warrant any pretreatment, even though it contained a significant level of oil (about 50-60 ppm). While pretreatment such as oil/water separation or solids removal may be needed in other cases, such decisions must be site and wastewater specific. Similarly, post-treatment decisions also depend on the specific site. At this facility, a decision was made to install a bag filter to collect the small amount of sloughed biomass that was anticipated, primarily so that pollutants in the sludge could be measured as part of the EPA investigation.

SITE TESTING PROGRAM

Working in collaboration with the developer of the process, it was determined that operation of the system at three increasing flow rates, 1, 3,

and 5 gpm, corresponding to residence times of 9, 3 and 1.8 hours, respectively, each for two weeks, would allow the effectiveness of the process to be determined at low contaminant loadings and at the design level. In fact, while the screening data reported in 1984 as part of the RI/FS had suggested high concentrations (~100-200 ppm) of pentachlorophenol might be present in the groundwater, when two wells were drilled in preparation for the project, a maximum of about 45 ppm pentachlorophenol and only low levels of polynuclear aromatic hydrocarbons (<1 ppm total PAHs) were found to be present.

The groundwater obtained from the selected well, the influent to, the effluent from, and the two intermediate stages of the bioreactor were monitored for pentachlorophenol, other semivolatile organics, chloride, and TOC. Chloride and TOC were monitored to provide supporting evidence for the vendor's claim that pentachlorophenol removal occurred by mineralization to water, carbon dioxide and salt by the following equation.



Other parameters also monitored to provide a complete history of the groundwater as it passed through the system included total and volatile suspended solids, oil and grease, nitrogen and phosphorus, volatile organics, and heavy metals. Because there is always concern when treating wastewaters containing chlorinated aromatics, testing was also done for chlorodioxins and furans.

Because this investigation was part of the SITE program and careful and complete analytical history (and safety) was desirable, carbon adsorption units were installed on both the aqueous discharge and on the air leaving the covered reactor chamber. Samplings and analyses were carried out before and after these units to determine whether significant quantities of the contaminants were lost by any route other than biodegradation.

Finally, static bioassays were carried out on the incoming groundwater, the influent to the reactor, and the effluent to learn whether the groundwater was toxic to aquatic species and whether treatment removed the chemical source of any toxicity.

RESULTS

From comparison of the pentachlorophenol concentrations for the groundwater as removed from the well and the effluent from the bioreactor, it is clear that the BioTrol system is capable of achieving about 96% removal of pentachlorophenol at the design flow rate, 5 gpm. And, at that flowrate, final effluent concentrations, before carbon polishing, are approximately 1 ppm. Table summarizes the pentachlorophenol removals at the three different flow rates.

TABLE 1. AVERAGE PENTACHLOROPHENOL REMOVAL BY THE BIOTROL AQUEOUS TREATMENT SYSTEM

Flow Rate (gpm)	Ground-water (ppm)	Effluent (ppm)	Percent Removal (%) Average	Range
1	42.0*	0.13	99.8 **	97.4-99.9+
3	34.5*	0.36	98.7 **	95.8-99.8
5	27.5*	0.99	97.6 **	79.3-99.4

* decrease with time may reflect drawdown of aquifer

** based on average of daily effluents

However, it must be noted that as the analytical results were obtained, it became apparent that an unexpected dilution phenomenon was occurring in the influent chamber where the composite influent samples were taken. The effect was a significant reduction in the apparent "influent" concentrations for pentachlorophenol (and other parameters) - and, presumably, in the values at the two intermediate sampling points as well. Where these values should have been essentially the same as the values for the groundwater, it was observed that they were considerably lower. Grab samples obtained by the vendor between the conditioning tank and the bioreactor and analyzed for pentachlorophenol using another method also confirmed the discrepancy. (In this alternate method, high pressure liquid chromatography (HPLC), the aqueous sample is injected directly onto a column at ambient temperature and the levels of pentachlorophenol measured with a UV detector at 254 nm and 220 nm. Although the method is not "EPA-approved" and was not subjected to the extensive quality assurance used for the GC/MS method, an abbreviated evaluation has demonstrated that the results are reliable and comparable to those obtained by GC/MS.) It is believed that the differences in concentrations, which were particularly significant at the lower flow rates, are the result of backmixing from each of the reaction chambers into the preceding mixing chambers. Consequently, the results being presented are based primarily on the incoming groundwater as it was analyzed at the well head and the final effluent from the bioreactor, using EPA Method 3510/8270, for which the Method Detection Limit for pentachlorophenol is 50 ug/L.

At the lower flow rates studied, 1 and 3 gpm, pentachlorophenol removals (based on the change from the groundwater to the effluent) increase to 99+% and final pentachlorophenol concentrations of 0.1 ppm and even less are achievable. These results are summarized in Table 1.

The changes in chloride and TOC results (weekly) parallel the decrease in pentachlorophenol at all flows (Table 2); however, they are not sufficiently precise to provide more than supportive evidence for mineralization of pentachlorophenol to sodium chloride, water, and carbon dioxide.

TABLE 2. COMPARISON OF AVERAGE CHLORIDE, TOC, AND PCP RESULTS

Flow Rate (gpm)	PCP	Change (delta) Cl(f) (ppm)	Cl(c)	TOC(f)	TOC(c)
1	-41.9	+44.2	-27.9	-24	-11.3
3	-34.1	+40.5	-22.7	-32	- 9.2
5	-26.5	+22.0	+17.6	-21	- 7.0

(f) = found; (c) calculated

As part of the effort to confirm that pentachlorophenol was being removed by biochemical mineralization and not by adsorption on the biosolids or by stripping due to the air in the bioreactors, both solids and air emissions were also monitored. Although the sludge trapped in the bag filter was found to contain pentachlorophenol (34 and 170 ppm found in two samples), the amount of sludge was so small that adsorption of pentachlorophenol on the biosolids and removal with the suspended solids does not represent a significant alternate removal mechanism. Thus, even if all the suspended solids (effluent - groundwater) produced by the system during the twelve days of the 1 gpm run were trapped in the filter, this would amount to only about 7 lbs of sludge. Even with a pentachlorophenol content as high as 170 ppm (which was measured in a later sample), this would only account for about 0.0012 lbs of PCP or about 0.02% of the total pentachlorophenol input of about 6.05 lbs. Similarly, pentachlorophenol was not present above the detection limit in any of the air samples obtained over the reactor chamber, using a modified Method 5 collection system with an XAD resin trap and an analytical method with a detection limit of 1.7 ug/cubic meter or 0.2 ppb. Therefore, it does appear that biological degradation is, by far, the primary means of eliminating the pentachlorophenol from the groundwater.

Concentrations of the various polynuclear aromatic hydrocarbons as part of the semivolatile fraction were below detection limits in the samples of incoming groundwater used in the demonstration program. Two analyses during the pre-demonstration testing indicated total PAHs of 145 and 295 ppb. Consequently, it is not possible to draw any conclusions as to removal efficiency or mechanism. However, several PAHs, including naphthalene and methyl naphthalene at maximum levels of 34.6 ppb and 47.9 ppb, respectively, and others at considerably lower levels, were found during the modified Method 5 testing of the air emissions from the reactor. This suggests that some air stripping of these constituents may be occurring.

Small amounts of various chlorinated dioxins were found in the effluent (<340 ng/L, using method SW8280) and, particularly, the sloughed biomass sludge, where one sample did exhibit 1900 ng/g of OCDD isomer. This value is currently being re-examined. With one exception, an effluent sample found to contain 62 ng/L, the 2,3,7,8-tetrachlorodioxin of primary concern was not detected in any of the influent, effluent, or sludge samples using high resolution GC coupled with low resolution MS.

The incoming groundwater was found to contain low concentrations of several

of the heavy metals, including nickel (<91 ug/L), zinc (<32 ug/L), copper (<25 ug/L), lead (<11 ug/L), and arsenic (<6.5 ug/L) from the chromated copper arsenate wood preservative currently used in wood treatment at the site. With the exception of one sample which is believed to be an anomaly, there was no change in the concentrations of the metals across the system.

Acute biomonitoring with fresh water minnows (96 hr static test) and daphnia magna (48 hr static test) demonstrated that the toxicity in the incoming groundwater or the influent was essentially totally removed by the treatment. LC50's increased from an estimated low of 0.2% (groundwater/control water) for the groundwater to more than 100% (as calculated from results) in the treated effluent.

COSTS

Preliminary cost estimates were carried out by the vendor for operation of the pilot plant at MacGillis and Gibbs excluding the ancillary equipment such as carbon units and bag filter but including cost for nutrients, electricity, heat, labor and caustic. In addition, costs were extrapolated by the vendor to a full scale system capable of treating 30 gpm of a similarly contaminated (~40 ppm pentachlorophenol) groundwater based on the demonstration study and other information at their disposal. On these bases, operating cost at the 5 gpm and the 30 gpm rate would be $4.24/1000 gallons and $2.62/1000 gallons, respectively (Table 3). As shown in the table, certain costs do not increase at the expected rate. For example, unit nutrient cost would decrease because of bulk purchase; electricity cost/gallon treated decreases because it is assumed that with deeper bioreactor beds in the 30 gpm unit (3 ft instead of 4 ft) the energy for the compressor supplying the air would be used more efficiently; operator labor cost also are not expected to increase in direct proportion to the size of the unit.

TABLE 3. OPERATING COST OF TREATMENT ($/1000 gal)

Cost Item	at 5 gpm	at 30 gpm
nutrients	0.042	0.017
electricity	0.416	0.216
heat	1.46	1.46
labor	2.08	0.69
caustic	0.24	0.24
TOTAL	4.24	2.62

These costs do not include leasing or amortization of the capital equipment, which are approximately $3,200/month (5 gpm mobile), $30,000 (5 gpm skid mounted) and $80,000 (30 gpm skid mounted), respectively.

Clearly labor and heat (electrical) requirements are the major factors to consider when treating waters at a specific site. And, of course, any site-specific pre- or post-treatment requirements, such as oil/water separation, solids removal, polishing, air emissions control, etc., would have to be factored into the cost calculation for that site.

CONCLUSIONS

On the basis of the pilot plant study carried out at the MacGillis and Gibbs site in Minnesota, the BioTrol process would be successful in treating groundwater or other pentachlorophenol-contaminated wastewaters (at ~40 ppm pentachlorophenol) to levels suitable for discharge to a POTW or reuse within a plant. One unforeseen benefit of the treatment was that biotoxicity in the incoming groundwater was eliminated by the treatment.

Contaminated waters of different concentrations can be accommodated by increasing or decreasing the throughput rate, recycling a portion of the stream or by sizing the system differently. Site-specific factors such as groundwater temperature, ambient temperature, extent of contamination with oil and/or solids, etc., can all play a role in the cost-effectiveness of overall treatment.

Although a secondary objective of the study was to evaluate the effectiveness of the system for removal of polyaromatic nuclear hydrocarbons that might be present at the site as a result of the use of creosote, the levels of these constituents in the groundwater used for the study were too low to reach any conclusions as to removal.

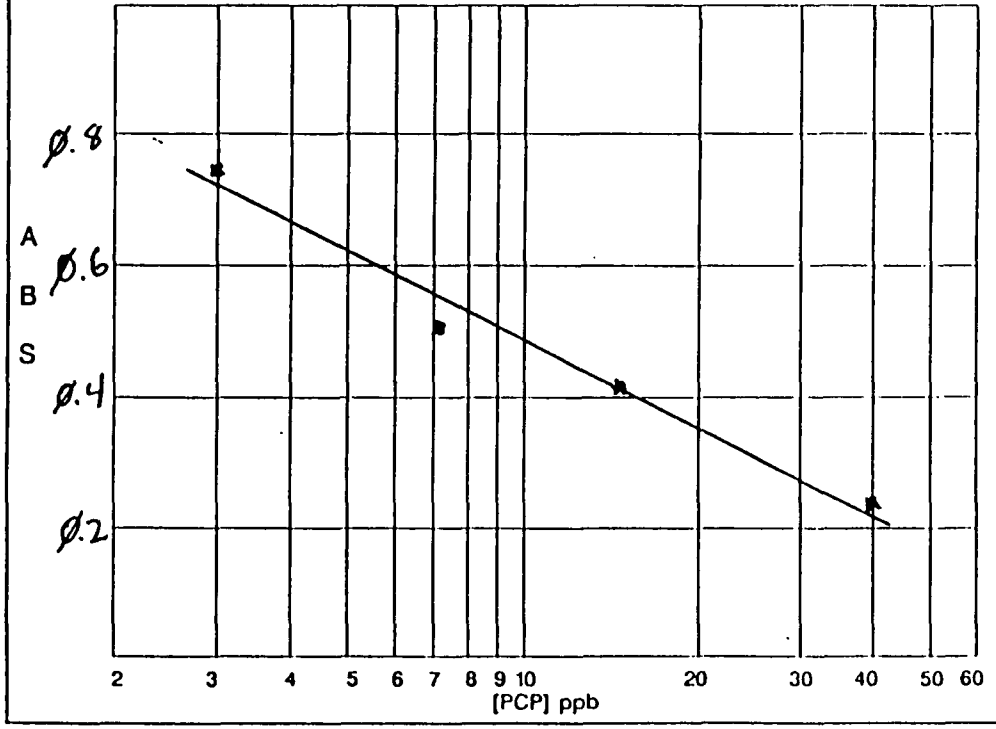
APPENDIX B

EXAMPLES OF KIT AND PLATE IMMUNOASSAY STANDARD CURVES AND SAMPLE PLACEMENT LAYOUT FOR THE PLATE IMMUNOASSAY

SAMPLE ANALYSIS INFORMATION BY STRIP

Sample number = ST1-A-Ø1-Ø5 / 25 JUL 89 / ON-SITE Strip ID: G-1

Sample Code		Well #	OD (ABS)	Plotted Conc (ppb)		to ppm		Dilution Factor		Sample Conc (ppm)	Amt(ppb) Spike Added	% Spike Rec
STDØ3	○	1	Ø.761	3.Ø	X	0.001	X	—	=	—.—	—.—	—.—
STDØ7	○	2	Ø.5Ø2	7.1	X	0.001	X	—	=	—.—	—.—	—.—
STD16	○	3	Ø.436	16.9	X	0.001	X	—	=	—.—	—.—	—.—
STD4Ø	○	4	Ø.267	4Ø.Ø	X	0.001	X	—	=	—.—	—.—	—.—
REMS-1	○	5	Ø.367	22.Ø	X	0.001	X	5Ø	=	1.1Ø	—.—	—.—
MSE-1	⊗	6	Ø.225	4Ø.Ø	X	0.001	X	—	=	—.—	15.Ø	12Ø
REMS-2	○	7	Ø.342	21.Ø	X	0.001	X	5Ø	=	1.Ø5	—.—	—.—
MSE-2	Ø	8	Ø.22Ø	41.Ø	X	0.001	X	—	=	—.—	15.Ø	133



Comments:

⊗ $\frac{40-22}{15} = \frac{18}{15} = 120\%$

Ⓥ $\frac{41-21}{15} = \frac{20}{15} = 133\%$

Figure B-1. Example of Field Data Form documentation for on-site kit immunoassay analysis during period A of the demonstration.

SAMPLE ANALYSIS INFORMATION BY STRIP

Sample number: ST1-B-Ø1-Ø2 / Ø7AUG89 / ON-SITE

Strip ID: D-1

Sample Code	Well #	OD (ABS)	Plotted Conc (ppb)		to ppm		Dilution Factor		Sample Conc (ppm)	Amt(ppb) Spike Added	% Spike Rec
STDØ3 ○	1	Ø.282	3.Ø	X	0.001	X	—	=	—•—	—•—	—•—
STDØ7 ○	2	Ø.232	7.1	X	0.001	X	—	=	—•—	—•—	—•—
STD16 ○	3	Ø.142	16.9	X	0.001	X	—	=	—•—	—•—	—•—
STD4Ø ○	4	Ø.Ø79	4Ø.Ø	X	0.001	X	—	=	—•—	—•—	—•—
RI ○	5	Ø.131	21.Ø	X	0.001	X	1000	=	21.Ø	—•—	—•—
RI ○	6	Ø.177	11.5	X	0.001	X	2000	=	23.Ø	—•—	—•—
RI ○	7	Ø.24Ø	4.8	X	0.001	X	4000	=	18.8	—•—	—•—
NC ○	8	Ø.624	—•—	X	0.001	X	—	=	—•—	—•—	—•—

Comments:

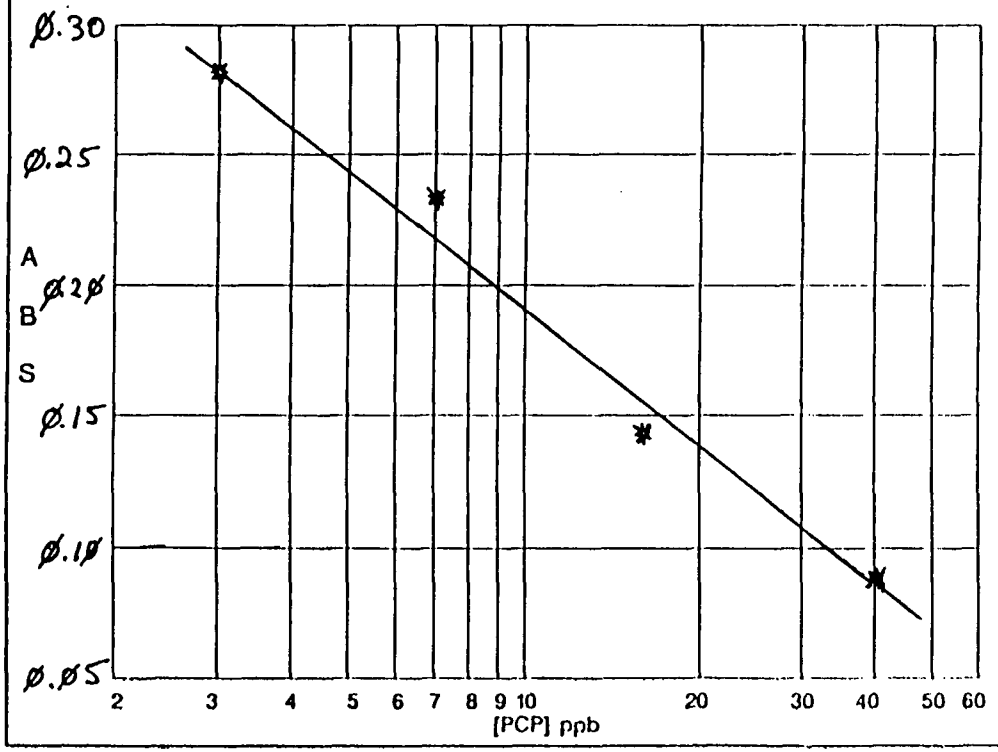


Figure B-2. Example of Field Data Form documentation for on-site kit immunoassay analysis during period B of the demonstration.

SAMPLE ANALYSIS INFORMATION BY STRIP

Sample number = QAA-C-ØØ-Ø1 / 23AUG89 / ON-SITE

Strip ID: B-2

Sample Code	Well #	OD (ABS)	Plotted Conc (ppb)		to ppm		Dilution Factor		Sample Conc (ppm)	Amt(ppb) Spike Added	% Spike Rec
STDØ3 ○	1	Ø.176	3.Ø	X	0.001	X	—	=	—•—	—•—	—•—
STDØ7 ○	2	Ø.164	7.1	X	0.001	X	—	=	—•—	—•—	—•—
STD16 ○	3	Ø.12Ø	16.9	X	0.001	X	—	=	—•—	—•—	—•—
STD4Ø ○	4	Ø.Ø74	4Ø.Ø	X	0.001	X	—	=	—•—	—•—	—•—
QAA-Ø1 ○	5	Ø.Ø92	26.Ø	X	0.001	X	1000	=	26.Ø	—•—	—•—
QAA-Ø1 ○	6	Ø.128	11.Ø	X	0.001	X	2000	=	22.Ø	—•—	—•—
QAA-Ø1 ○	7	Ø.134	8.Ø	X	0.001	X	4000	=	32.Ø	—•—	—•—
NC ○	8	Ø.166	3.5	X	0.001	X	—	=	Ø.ØØ3	—•—	—•—

Comments:

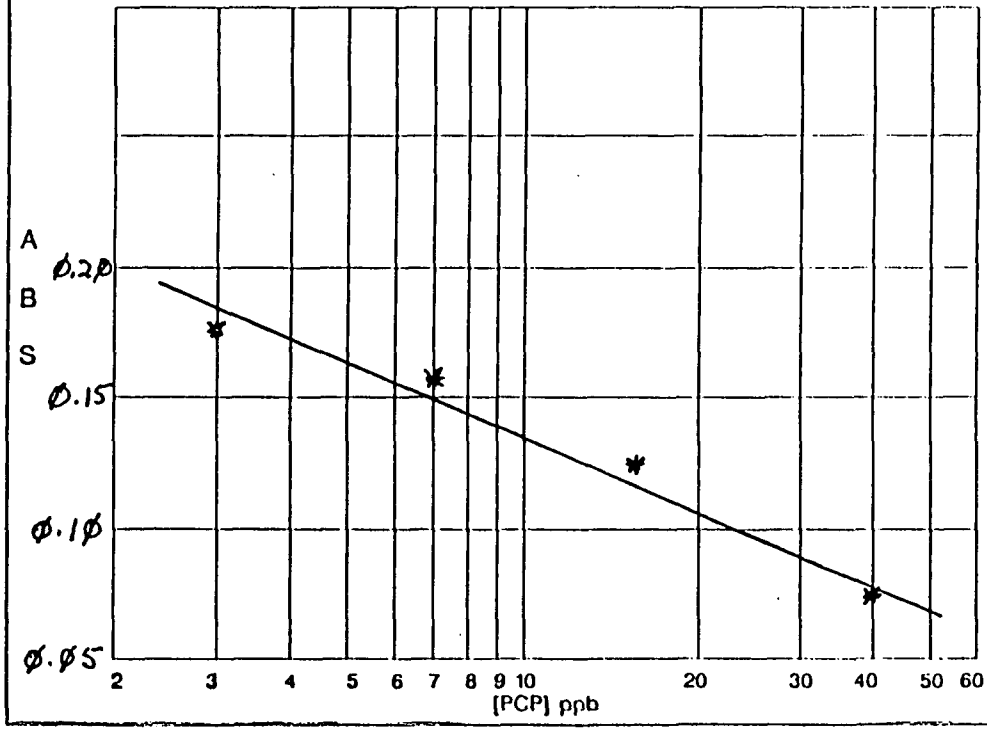


Figure B-3. Example of Field Data Form documentation for on-site kit immunoassay analysis during period C of the demonstration.

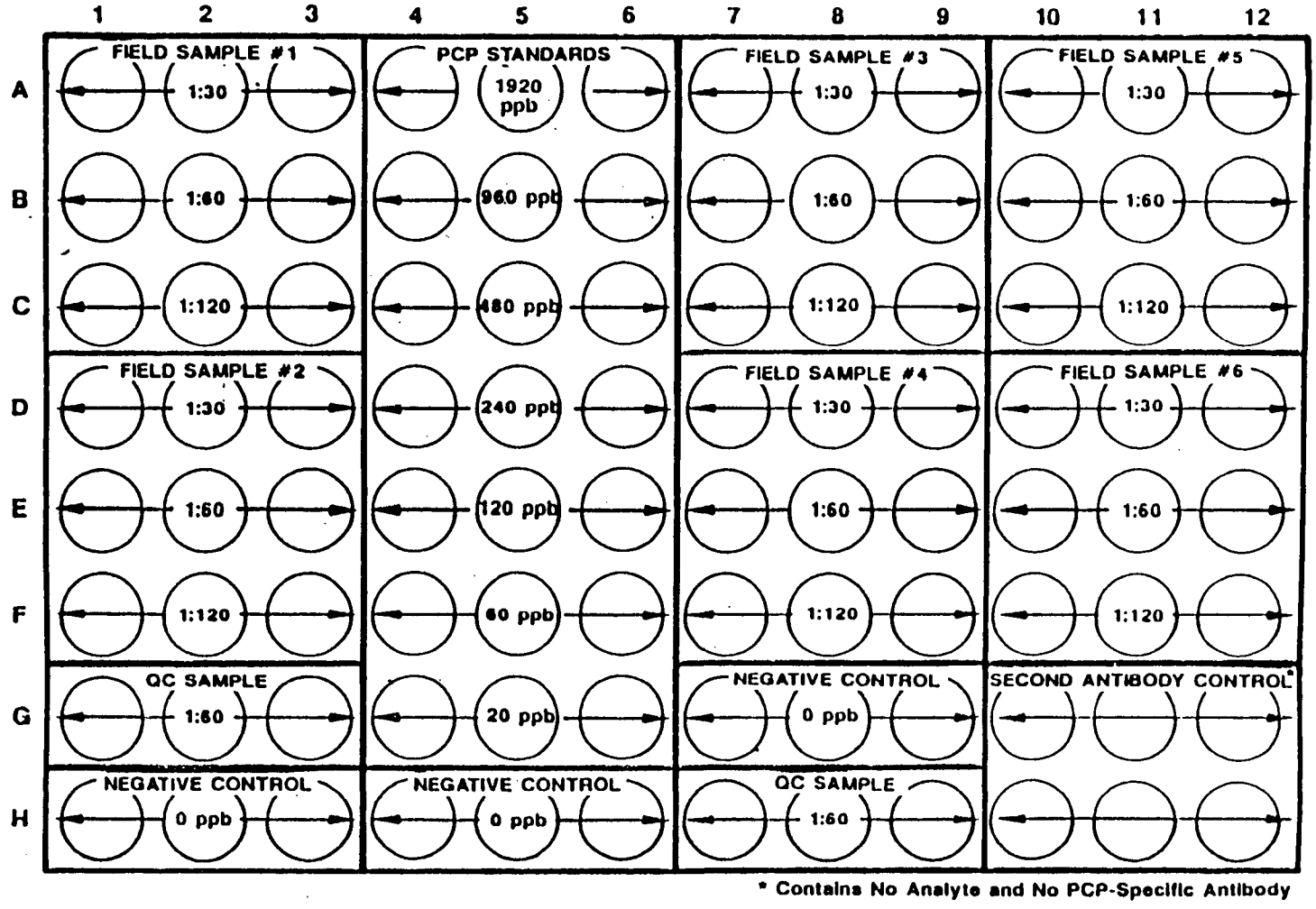
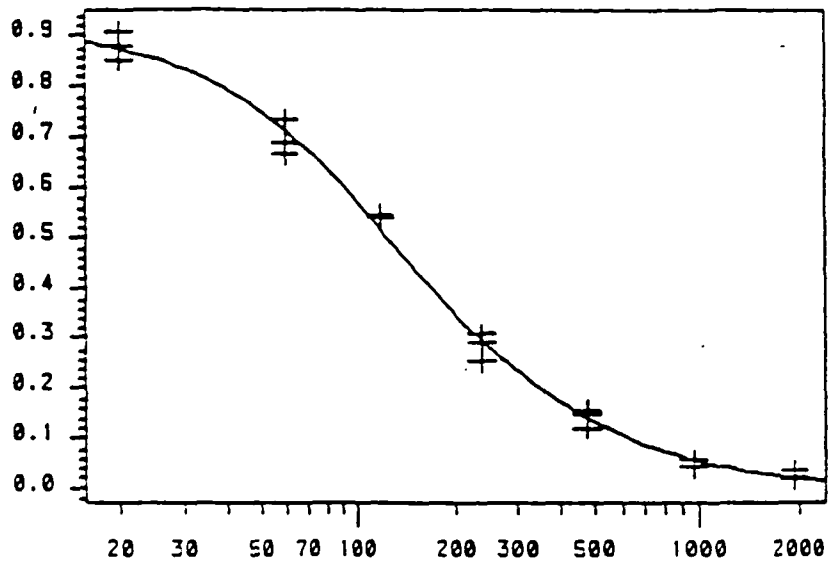


Figure B-4. Example of the sample placement layout on a typical plate immunoassay analyzed in the SITE demonstration.



Optical Density vs. Concentration, logarithmic (ppb)

Fitting Method:

Four-Parameter

Regression Parameters:

a = -0.0031

b = -1.4085

c = 4.9438

d = 0.9301

R-sqr = 0.9913

Sterr = 0.0240

Calculated Concentrations:

Concentration at mid-point of O.D. range = 140.3052

Concentration at 10% of O.D. range = 667.6631

Concentration at 90% of O.D. range = 29.4842

Figure B-5. Example of a typical standard curve generated for the plate immunoassay.

APPENDIX C

DATA QUALIFIER FLAGS AND DEFINITIONS APPLIED TO KIT IMMUNOASSAY DATA DURING DATA VERIFICATION

Flag

A = Definition: data point (sample concentration) inconsistent with other dilutions on the same strip.

Application: This flag only applies to influent and effluent sample range-finding data or to audit sample (QAA, QAB) data. If optical densities of range-finding strips of one or all serial dilutions analyzed on a particular strip did not follow in a logical order (i.e., the greater the dilution the higher the absorbance units), then an A flag was applied to the dilution(s) (11 of 104 range-finding strips had this occurrence across all analysis sites). Field sample (influent, effluent, raw influent) concentrations with A flags were not used when determining the concentration of the sample for intra- and intermethod comparisons (see Appendix D). On the other hand, QAA and QAB audit samples that had A flags applied to them (5 occurrences out of 36 strips) were included in the statistical analyses of such performance parameters as precision, accuracy, and false negatives.

B = Definition: inconsistent calibration standard, data point not used in calculation of sample concentration.

Application: This flag was applied to one of the four PCP calibration standards (3.0, 7.1, 16.9, or 40.0 ppb) when a straight line could only be drawn through the other three. Of the 256 strips used, 25 had calibration curves with one inconsistent standard (see Section 5 for details, and for computer versus hand-calculated results). There was never more than one B flag per strip. If a straight line could not be drawn using at least three calibration standards, no B flag was applied. See the description for D flags for a discussion on when more than one calibration standard was inconsistent.

C = Definition: illegible or omitted number entry or value reported - number critical in calculating sample concentration.

Application: The C flag was only applied to one calibration standard (out of more than 1,000) that was illegible and to one sample for the dilution factors of a set of analyses.

D = Definition: all calibration curve data suspect, no confidence in any sample data generated from this strip.

Application: When a straight line could not be drawn between at least three of the four calibration standards, or when the optical densities of the standards were not acting in an expected fashion (i.e., the lower the ppb standard, the higher the optical density), the calibration curve was considered erratic. As a result, these results were not used for any further analysis. Of the 274 calibrations used in the demonstration, 18 had this occurrence (see Section 5 for a discussion).

E = Definition: operator-noted analytical problem with the analysis in the particular well.

Application: This flag was reserved for standards and samples for which comments on the field forms indicated analytical problems such as reagents omitted from analyses or bubbles

observed in the well. These data points were not used in any sample or statistical calculations. There were only 6 of these occurrences, representing less than 0.2% of the kit immunoassay data.

F = Definition: inconsistent matrix spike-related result.

Application: These flags were applied to matrix spike samples exhibiting grossly poor (and negative) spike recovery results. Of the 82 spiked samples, 7 have F flags applied to them. See Section 5 for the discussion of matrix spike performance.

G = Definition: deviation from standard protocol, strip results reported twice.

Application: For only one strip, the sample results were reported twice by the analyst. This is a deviation from standard protocol, and the second set of results were not used in any statistical analyses because results would be improperly weighted.

ZZ = Definition: sample result used in the determination of PCP concentration to represent the sample in intra- and intermethod comparisons.

Application: Sample results with the ZZ flag for each sample were used in the comparisons of kit to plate immunoassay and immunoassay to GC/MS methods. For each sample, if there was more than one result with a ZZ flag applied to it, the average was taken to determine the PCP concentration to be used in the comparisons (see Appendix D for a detailed explanation). NOTE: These flags were also applied to plate immunoassay and GC/MS samples for the method comparisons.

APPENDIX D

ALGORITHMS USED TO DETERMINE PENTACHLOROPHENOL CONCENTRATIONS IN SAMPLES USED IN METHOD COMPARISONS OF THE KIT AND PLATE IMMUNOASSAYS

Kit Immunoassay

Samples were analyzed by the kit immunoassay method according to specific procedures in order to yield a variety of performance and concentration data for each sample. Five field kit strips per sample, resulting in ten or more "valid" sample concentration results, were not unusual. As a result, it was difficult to determine which sample concentration would "represent" each sample, an issue that was not adequately considered in the demonstration or quality assurance project plans. In an effort to select fair and unbiased sample concentrations for intra- and intermethod comparisons, sample concentrations were chosen based on an approach that might be used by a field analyst who had little or no prior knowledge of the amount of analyte expected.

Specifically, an analyst using the kit immunoassay would first perform a range-finding (screening) step in order to ascertain whether there was any detectable pentachlorophenol (PCP) and, if so, which dilution would bring the analysis within the linear (calibration) range of the method. After the proper range was estimated, replicate analyses could be conducted at that optimum dilution. With this background, the following logic was used to select the PCP concentration for each kit immunoassay sample used in the method comparison analyses:

1) If a duplicate or method split strip was run, the average from the first pair was used. (For influent samples the mean of the RI (routine influent) and SRI (split of routine influent) samples was used; for effluent samples the mean of RE (routine effluent) and SRE (split of routine effluent) samples was used.

2) If no duplicate or method split analyses were available for the sample and a matrix spike strip was run for the sample, then the average from the pair of "unspiked" samples (i.e., mean of the RIMS-1 and RIMS-2 and the mean of effluent samples coded REMS-1 and REMS-2) was used. (NOTE: RIMS and REMS are matrix spike "pre-spike" samples for influent and effluent samples; the numbers 1 and 2 refer to the two spikes per strip).

3) Samples were reanalyzed after the original analysis day because of either a request resulting from the EMSL-LV QA data review or an indication that the results seemed suspicious for various documented reasons (e.g., bad calibration curves, presence of bubbles in the wells, or a preconceived expectation by the operator of the sample concentration or the optical density). It is standard QA practice to report reanalysis results instead of original analysis results if the original results are suspicious and the reanalyzed results are considered sound and valid. In these cases, the same rationale for steps 1 and 2 were used to determine sample concentration, as appropriate.

4) If, after performing the previous 3 procedures, no appropriate sample concentration could be determined, then the average of the two lowest dilutions from the range-finding strip was used. If only one dilution was in range, then only that concentration was used. The less diluted samples were used to minimize possible errors and variability associated with sample dilution, which can increase with the number of dilution steps. (A separate statistical analysis of dilution variability was performed using a variety of sample types and dilution factors; see Section 5.) In addition, whenever steps 1, 2, and 3 yielded sample values that were diluted below detectable

limits, a quantified concentration was chosen for the sample from another source (e.g., the range-finding strip).

5) If, when selecting a PCP concentration based on range-finding strip results, there was a large discrepancy in the two lowest dilutions (i.e., by a factor of 2 or more), the two most "consistent" numbers were picked if they existed on the same or another strip. For example, when there was 4.2, 42.0, and 36.0 ppm for 1:100, 1:1,000, and 1:2,000 concentrations, respectively, the 42.0 and 36.0 were averaged even though the 4.2 was the lowest dilution.

Plate Immunoassay

The selection of the PCP concentrations for the plate immunoassay in the intra- and intermethod comparisons was based on *a priori* knowledge of immunoassay performance and data quality components (e.g., calibration curve linearity). The "best possible" plate concentration was determined by averaging all the available laboratory data for each sample. This procedure differs markedly from those used to determine the PCP data analyzed by the kit immunoassay, which was based on the probable approach a field technician would normally take during a site investigation. The most accurate concentration for the plate immunoassay is desirable for the rigorous comparison of the strip method. Hence, the plate immunoassay results are based on more QA, QC, and range-finding sample results than would be typical in a normal, production-oriented, sample analysis mode by this method.

The following logic was used for sample concentration selection for the plate technique:

1) Based on the fact that the most accurate sample concentration for a particular analysis can be obtained from the linear portion of the standard calibration curve, samples diluted into this range (approximately 50 to 550 ppb) for the plate immunoassay were included as data to be pooled and averaged for the concentration of that sample. In other words, any range-finding, duplicate, split, or matrix spike (unspiked portion) analysis generating sample data within this 50 to 550 ppb range was considered a reliable and defensible value to be used in determining the best estimate of the sample concentration. After these analyses were selected, all of the concentrations were averaged to estimate the concentration for that sample for each analysis site.

2) If none of the plate concentration analyses fell within the 50 to 550 ppb range, the average of all analyses for the sample outside of the range was used. Although these results may not be considered as accurate or reliable as those within this range, they were chosen because they were still the best possible values available for the particular sample.

3) The EMSL-LV laboratory had difficulty with the initial range-finding analyses by the plate immunoassay method. The set of reagents used in these analyses generated standard curves that were markedly off-set from the ranges expected, indicating that the immunoassay conditions were not optimized. After completing the range-finding analyses on all the field samples (on September 17, 1989), another set of reagents was supplied to EMSL-LV by WBAS. These reagents were titered to determine optimal

levels. EMSL-LV plate immunoassay analyses that were performed with the new set of reagents had standard curves in the expected concentration range. Subsequently, the duplicate, split, and spike sample analyses were performed and almost all of the samples originally analyzed on range-finding strips were reanalyzed. Data generated after September 17, 1989 were selected, when available, instead of initial results obtained from the first set of reagents.

APPENDIX E

PARTICIPATING PERSONNEL

Many individuals from various organizations contributed to the success of the WBAS immunoassay demonstration and participated in the preparation of this report. Their names, organizations, and contributions are provided below.

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