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Superfund Innovative Technology Evaluation Program Demonstration Plan for Westinghouse Bio-Analytic Systems Pentachlorophenol Immunoassays

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SUPERFUND INNOVATIVE TECHNOLOGY EVALUATION PROGRAM DEMONSTRATION PLAN FOR WESTINGHOUSE BIO-ANALYTIC SYSTEMS PENTACHLOROPHENOL IMMUNOASSAYS

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NOTICE

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ABSTRACT

This plan provides a detailed design and description of the demonstration and evaluation program for the Westinghouse Bio-Analytic Systems immunoassay technologies specific for the analysis of pentachlorophenol. The immunoassays measure parts per billion concentrations of pentachlorophenol in water. This demonstration is being conducted under the Superfund Innovative Technology Evaluation (SITE) Program.

The main focus of this demonstration is to evaluate on site a semiquantitative immunoassay field analysis kit for its utility as a rapid field screening tool. The results obtained from the field kit analyses will be compared to those obtained from a quantitative high-sample-capacity plate immunoassay also developed by Westinghouse Bio-Analytic Systems. In addition, both immunoassay techniques will be compared to the standard gas chromatography/mass spectrometry procedure for pentachlorophenol determination. The demonstration will be 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 will be performed in tandem with a SITE demonstration of a bioremediation technology (a bioreactor developed by BioTrol, Inc.), that is designed to biodegrade pentachlorophenol in water.

The demonstration plan provides the sampling plan and design specific to the immunoassay technologies demonstration, including testing duration, test site description, logistical and equipment considerations, communications between analysis and management locations, sample collection and handling protocols, sample identification and tracking systems, and chain-of-custody and sample shipping procedures. The quality assurance plan for this demonstration is provided in Appendix A. The demonstration will enable data users and reviewers to assess the performance of the technology in terms of its usefulness and limitations for the Superfund Program.

This plan is submitted in partial fulfillment of contract number 68-03-3249 and number 68-CO-0049 by Lockheed Engineering & Sciences Company under the sponsorship of the U.S. Environmental Protection Agency. The development of this plan began in May 1989. The plan was implemented from July through September of 1989 for the field and laboratory operations.

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ABBREVIATIONS AND ACRONYMS

AFMMP	Advanced Field Monitoring Methods Program
ANOVA	analysis of variance
cc	cubic centimeter
CLP	Contract Laboratory Program
CFR	Code of Federal Regulations
DCP-THY	dichlorophenol conjugated with thyroglobulin
DOT	U.S. Department of Transportation
EPA	U.S. Environmental Protection Agency
EMSL-LV	Environmental Monitoring Systems Laboratory - Las Vegas, Nevada
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
id	identification
L	liter
LESC	Lockheed Engineering & Sciences Company
M	molar
mL	milliliter
mM	milliMolar
mm	millimeter
MSDS	Materials Safety Data Sheet
N	Normal
nm	nanometer
NPL	National Priorities List
PAH	polyaromatic hydrocarbons
PCP	pentachlorophenol
POTW	publicly owned treatment works
ppb	parts per billion
ppm	parts per million
QA	quality assurance
QAPjP	Quality Assurance Project Plan
QC	quality control
RREL	Risk Reduction Engineering Laboratory
RSD	relative standard deviation
SAIC	Science Applications International Corporation
SARA	Superfund Amendments and Reauthorization Act of 1986
SAS	Statistical Analysis System
SITE	Superfund Innovative Technology Evaluation
SOP	standard operating procedure
WBAS	Westinghouse Bio-Analytic Systems
µL	microliter

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EXECUTIVE SUMMARY

This demonstration plan has been prepared under the Superfund Innovative Technology Evaluation (SITE) Program and provides a detailed design and description of the SITE demonstration of the Westinghouse Bio-Analytic Systems (WBAS) immunoassay technologies specific for the analysis of pentachlorophenol. The immunoassays measure parts per billion concentrations of pentachlorophenol in environmental water samples.

The primary objective of this demonstration is to evaluate on site a semiquantitative immunoassay field kit for its utility as a rapid field screening tool. This demonstration plan provides the design and protocols required to obtain the information needed for this evaluation.

The field kit will be compared to a quantitative high-sample-capacity plate immunoassay developed by WBAS that was previously evaluated at the U.S. Environmental Protection Agency (EPA), Environmental Monitoring Systems Laboratory, Las Vegas, Nevada (EMSL-LV). Both of these immunoassay techniques will be compared to standard EPA methods used for the analyses of pentachlorophenol in water by gas chromatography/mass spectrometry. The demonstration will be conducted at the MacGillis & Gibbs Superfund Site in New Brighton, Minnesota. This is a National Priorities List site known to have ground water contaminated with pentachlorophenol. The immunoassay demonstration will be performed in tandem with a SITE demonstration of a bioremediation technology (a bioreactor developed by BioTrol, Inc.) that is designed to biodegrade pentachlorophenol in water.

Immunoassays are based on receptor molecules called antibodies which are developed in response to a particular target analyte. Quantification of the extent of contamination in an environmental sample is based on the ability of a specific antibody to bind to its target analyte. Immunoassays are normally based on competition for antibody binding between a known amount of analyte labeled with an indicator, such as an enzyme, and an unknown amount of analyte from a sample. The indicator produces a colored product that is used for quantitation. Color intensity is determined by the amount of analyte present. Immunoassays can be configured for quantitative, semiquantitative, or qualitative analyses.

The immunoassay field kit methodology requires about 30 minutes to perform and has a detection limit of about 3 parts per billion (ppb). It has a linear dynamic range from about 3 to 40 ppb and uses no more than 2 milliliters (mL) of sample to obtain analytical results. The methodology employs a portable spectrophotometer for standard curve generation and quantification of pentachlorophenol concentrations and it requires a clean, sheltered work area (e.g., out of the wind and direct sunlight).

The quantitative (i.e., plate) immunoassay is based on a 96-well microtiter plate format. The minimum detectable level of pentachlorophenol is approximately 30 ppb; however, when the required sample dilutions are considered, the minimum detection level is 90 ppb, based on an 18-mL sample. The method has a linear dynamic range of 30 to 400 ppb. Several 96-well microtiter plates can be processed in tandem. Thus, hundreds of samples can be analyzed during the 2.5-hour analysis time required for this method. The plate immunoassay also can be performed under field conditions similar to those required for the field kit. However, for this demonstration, only the field kit will be evaluated under field conditions because this methodology is particularly suited for use by field personnel who may have limited analytical chemistry experience.

This demonstration plan provides the specific sampling plan and design for the immunoassay technologies demonstration, including testing duration, test site descriptions, logistical and equipment considerations, communications between analysis and management locations, sample collection and handling protocols, sample identification and tracking systems, and chain-of-custody and sample shipping procedures. A detailed quality assurance plan for this demonstration is provided in Appendix A.

The sample collection, sample analysis, and data analysis strategies presented in this plan are designed to address the critical issues related to assessing the general and practical applications of immunoassay technology in the measurement and monitoring aspects of the Superfund Program. The results obtained from samples analyzed on site during the demonstration of the field analysis kit immunoassay technique will be compared to results obtained by analyzing splits of the same samples. For this comparison, standard EPA sample preparation and analysis methods for determining pentachlorophenol in water by gas chromatography/mass spectrometry will be used. Split field samples will also be analyzed off site by both immunoassay techniques (field kit and plate) at EMSL-LV and WBAS laboratories.

The demonstration plan includes detailed standard operating procedures for sample analysis and data management. The quality assurance plan is designed to ensure that important data quality and methodological performance criteria are examined. A series of performance evaluation (audit) samples, as well as blank and replicate samples, are incorporated in the analytical scheme to assess the within-method performance parameters of the immunoassay and to perform between-method comparisons. The data management system is tailored to the sample analysis and quality assurance programs to provide a timely means of performing the data analysis. Data management will also provide a mechanism for documenting and tracking the data generated from the different analysis sites and by the various methods.

As pentachlorophenol is considered a toxic substance and is a suspected carcinogen, the plan addresses health and safety aspects associated with handling and disposing of materials contaminated with pentachlorophenol.

It is anticipated that a fair performance evaluation of the pentachlorophenol immunoassay technologies can be determined by following the specifications in this demonstration plan. Thus, upon completion of the demonstration, data users and reviewers will be able to assess the performance of the particular pentachlorophenol immunoassays in terms of their utility and limitations in the Superfund Program.

1. INTRODUCTION

This demonstration plan provides the specific sampling and analytical design for the evalution of a semi-quantitiative immunoassay technique used for the rapid detection of pentachlorophenol (PCP) in environmental water samples. The demonstration of this field screening technology, developed by Westinghouse Bio-Analytic Systems (WBAS), will be conducted under the Superfund Innovative Technology Evaluation (SITE) Program.

1.1 OVERVIEW OF THE SITE PROGRAM

The Superfund Amendments and Reauthorization Act of 1986 (SARA) charged the U.S. Environmental Protection Agency (EPA) with effecting more timely and cost-effective remedies at the Nation's Superfund sites. The costs incurred for site characterization are a direct result of sampling, analysis, and the associated quality assurance activities. If field screening methods can yield immediate or short-turnaround environmental data, the use of these methods will result in major cost savings. The cost-effectiveness of clean-up efforts will be improved dramatically. More cost-effective and timely remediation will decrease the human and ecological risks around Superfund sites and enhance the ability to manage such risks.

The EPA SITE Program was established to satisfy the mandate in Section 311(b) of SARA, which requires the EPA to establish "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." The two categories of technologies included in the SITE Program are (1) treatment technologies that may serve as alternatives to land disposal of hazardous wastes and (2) monitoring and measurement technologies for contaminants occurring at hazardous waste sites. The Monitoring and Measurement Technologies Program is that component of SITE established to address the second category.

The SITE Program provides the Agency with a good mechanism to identify and demonstrate innovative or alternative site characterization technologies, existing within and outside the Federal government, which may provide cost-effective, better, and faster means to detect and monitor contaminants at uncontrolled hazardous waste sites. This Program also provides developers with the means to rigorously evaluate the performance of their technologies and have the results and recommendations widely distributed, thereby enhancing the market for those technologies.

Products from the various research, development, and demonstration activities conducted under this Program will enhance the Agency's ability to perform statistically valid sampling and field analytical programs that yield effective site characterization coupled with immediate or quick-turnaround environmental data acquisition.

The Monitoring and Measurement Technologies Program portion of SITE is also the core of the Advanced Field Monitoring Methods Program which was implemented in fiscal year 1988 to provide a mechanism to identify, test, evaluate, and accelerate the use of innovative and alternative field monitoring and measurement technologies, primarily in support of the Regional Superfund staffs. The Advanced Field Monitoring Methods Program enhances the SITE Program by adding an in-house methods research element and additional technology transfer through the preparation, testing, and promulgation of standard methods and the development of protocols for the successful use of technologies by field personnel.

The process of selecting and accepting measurement and monitoring technologies into the SITE demonstration program begins by compiling a list of candidate technologies identified through a variety of sources. The technologies are then screened and assessed for anticipated performance and application to Superfund site characterizations and for their ability to meet one or more of the following criteria:

- can be used on-site (outdoors or in a mobile laboratory);
- is widely applicable to a variety of sites;
- offers a high potential for solving critical problems for which current approaches are less than satisfactory;
- costs significantly less than current methods;
- has significantly better performance than current methods (e.g., better data quality, faster sample preparation or analysis time); and
- uses techniques that are easier and safer to perform than current methods.

Once the technology has been accepted into the SITE Program, the developer and the EPA will enter into a series of negotiations and planning discussions intended to culminate in a rigorous and comprehensive field demonstration to evaluate the utility of the technology. Activities required to prepare and execute of a demonstration are described in this plan.

After on-site demonstration activities are completed, a detailed and thorough evaluation of the demonstration results and comparisons will be fully documented in an evaluation report. This report will be prepared by the EPA and reviewed by Agency and non-Agency experts for independent assessment and peer review of the results and conclusions presented.

1.2 IMMUNOASSAY PROGRAM DESCRIPTION

Immunoassays are increasingly being recognized as cost-effective alternatives to chromatographic and spectroscopic procedures for use in rapid, large-scale environmental monitoring studies. Immunoassay techniques have been applied to the analysis of many hazardous substances and possess several attributes that make them suitable for field screening methods. In general, immunoassays have proven to be sensitive, selective, precise, rapid, cost-

effective, and applicable to a wide range of contaminants. Several different immunoassay formats for environmental chemistry are possible.

Identifications of immunoassays of Agency interest are sought from private industry and academia through solicitations in scientific journals and the *Commerce Business Daily*. These assays are evaluated and, if appropriate, adapted for Agency use. Immunoassays have been applied to many compounds and compound classes which are of interest to the EPA.

Although specific immunoassays have been developed for hazardous compounds, many of these systems have not been configured to analyze real-world environmental samples. Agency restraint in utilizing immunoassay technology is partially due to the lack of fully developed methods for environmental matrices. Demonstration that an immunoassay is properly developed will lead to implementation of the technology into environmental monitoring and exposure assessment studies. Thus, the Agency would be supplied with rapid, low-cost monitoring capability for compounds difficult to analyze by conventional methods.

Specific immunoassay methods that have been developed for a particular environmental application are submitted to EMSL-LV for characterization and evaluation. Such testing ensures that the intended environmental application of the method is appropriate. According to Agency guidelines for methods evaluations, this process requires the determination of such performance parameters as precision, within- and among-laboratory bias, between-method bias, method detection limits, matrix effects, interferences, limit of reliable measurement, and ruggedness of the method. Real-world samples are used when available, particularly when the immunoassay program can coordinate analytical support with other concurrent Agency studies. Analytical results generated from the immunoassay technique can then be compared to those results obtained from existing (e.g., Contract Laboratory Program [CLP]) analytical methods.

If a particular immunoassay is of the appropriate design and quality, based upon a preliminary laboratory evaluation of the method, a SITE demonstration may be conducted. Specific descriptions of the immunoassay techniques used in this SITE demonstration can be found in Section 2 and in the Quality Assurance Project Plan (QAPjP) (Appendix A).

1.3 OVERVIEW OF THE SITE DEMONSTRATION FOR THE WBAS PENTACHLOROPHENOL IMMUNOASSAYS

The technologies for which this demonstration plan has been prepared are a semiquantitative immunoassay field analysis kit and a quantitative 96-well microtiter plate immunoassay for the rapid screening of PCP in aqueous samples. The technologies were developed by Westinghouse Bio-Analytic Systems (WBAS) of Rockville, Maryland. The methods are designed to provide a quick and inexpensive means of detecting PCP in water under field or mobile laboratory conditions. The major focus of the SITE study is to demonstrate and evaluate the field analysis kit. The quantitative (plate) assay has already undergone an extensive laboratory evaluation at EMSL-LV (Section 2.4). The quantitative assay can be easily performed under conditions similar to those required for the field analysis kit (i.e., field laboratory).

An opportunity is available to coordinate the WBAS immunoassay demonstration with the field and analytical operations of another planned SITE demonstration that will evaluate a technology for use in Superfund site remediations. Specifically, BioTrol, Inc., of Chaska, Minnesota, has developed a biological reactor designed to degrade PCP (as well as polyaromatic hydrocarbons) into carbon dioxide, water, and inorganic chloride.

The performance of the BioTrol bioreactor will be demonstrated at the MacGillis & Gibbs site located in New Brighton, Minnesota. The MacGillis & Gibbs site, which is on the EPA National Priorities List (NPL), contains ground water contaminated with PCP as the result of a wood preservative treatment operation. The PCP-contaminated ground water will be pumped into the bioreactor and discharged as treated effluent (Appendix B). Preliminary data, as described in Appendix B, show that the ground water from the well supplying the bioreactor influent contains about 50 ppm PCP and the treated effluent contains about 1 ppm. Composite samples will be collected once every 24 hours for a period of 6 weeks at various critical points in the bioreactor's system, including the conditioned (i.e., pH-adjusted and nutrient-added) ground-water influent and the fully treated effluent. These samples will be processed and analyzed for a variety of organic, inorganic, and physical characteristics, including the use of gas chromatography/mass spectrometry (GC/MS) analysis for PCP (EPA Method 8270 after Method 3510 extraction). The complete description of the BioTrol bioreactor technology and its demonstration under the SITE Program can be found in the bioreactor demonstration plan (SAIC, 1989).

This bioreactor demonstration presents an excellent opportunity to simultaneously test the effectiveness of the remediation technology and the measurement and monitoring technology, while minimizing logistical and analytical costs. The QAPjP for the immunoassay demonstration (Appendix A) has been designed to ensure the collection of enough data to make the necessary methodological and statistical comparisons between the WBAS immunoassays and an established GC/MS method and to assess the quality of the data acquired using the immunoassays, especially the field kit.

Although the primary goal of this demonstration is to compare the field immunoassay PCP data to those obtained by GC/MS, both immunoassays will be compared to each other and the plate immunoassay results will also be compared to GC/MS results obtained from analysis of split samples. In previous studies, data from the plate immunoassay have been compared to data from GC analyses. Section 2.3.2 describes the plate immunoassay technology and Section 2.4 presents a discussion of the results of those previous studies. This demonstration is expected to complement and supplement the previous studies. The comparisons of these three methods (field kit, plate, GC/MS) will maximize performance information on measurement and monitoring technologies with a minimum of time, cost, and resources.

NOTE: Issues relevant to the performance and the evaluation of the bioreactor demonstration are not within the scope of this document, nor within the scope of the WBAS field kit demonstration, and will be documented elsewhere.

1.4 PURPOSE OF THIS DEMONSTRATION

The main purpose of this demonstration is to evaluate a rapid, on-site immunoassay technique for the detection of PCP in environmental water samples. Preliminary performance data from

testing in a controlled laboratory environment have been generated by using specified concentrations of PCP spiked into laboratory-grade water. In addition, these tests included analyses of various natural and bioreactor matrix water samples (more detail is provided in Section 4). This field demonstration provides the opportunity to assess the ruggedness of the field kit immunoassay technology under field conditions using environmental samples known to be contaminated with PCP. The same water samples used to test the WBAS field kit will be analyzed for a suite of other chemical, physical, and biological parameters (see Appendix C for these analytes and methods). Therefore, these analyses present an opportunity to assess site-specific interferences. Comparisons will be made among the data generated from the field kit immunoassay performed on- and off-site, the laboratory immunoassay, and the GC/MS results.

The following elements will be addressed and implemented in the demonstration:

- site and logistical considerations and support (coordinating with another demonstration to minimize necessary costs, efforts, and resources);
- sampling and analysis plan;
- quality assurance project plan;
- data handling and analysis plan; and
- health and safety plan.

These elements are discussed in greater detail in the following sections.

1.5 PARTIES INVOLVED AND RESPONSIBILITIES

The responsibilities of the EPA Environmental Monitoring Systems Laboratory in Las Vegas, Nevada (EMSL-LV), with assistance from its prime contractor, Lockheed Engineering & Sciences Company (LESC), for the demonstration of the WBAS immunoassays include:

- designing, overseeing, and implementing the elements of this demonstration plan;
- acquiring the necessary confirmatory data; and
- evaluating and reporting on the performance of the technology.

WBAS, the developer of the immunoassays being demonstrated, is responsible for:

- providing technical assistance to the personnel using the field kit on-site,
- performing immunoassay analyses of split samples, and
- supplying a sufficient number of field kits and laboratory reagents (for the plate immunoassay) to fully satisfy the requirements set forth in this demonstration plan.

The main function of the Risk Reduction Environmental Laboratory (RREL), through its prime contractor, Science Applications International Corporation (SAIC), Paramus, New Jersey, is to perform the BioTrol bioreactor demonstration. Aspects of the immunoassay demonstration for which RREL and SAIC are responsible include:

- performing the field immunoassay on the prescribed water samples on site;
- providing the logistical support designated in this plan; and
- analyzing the influent and effluent samples by GC/MS and reporting the results.

For the immunoassay demonstration, BioTrol, Inc., the developer of the bioreactor being demonstrated as a remediation technology, is responsible for:

- providing predemonstration test samples and
- providing technical assistance.

Figure 1.1 shows the organizational structure and key personnel. Specific task responsibilities for all of these parties are detailed in the QAPjP (Appendix A).

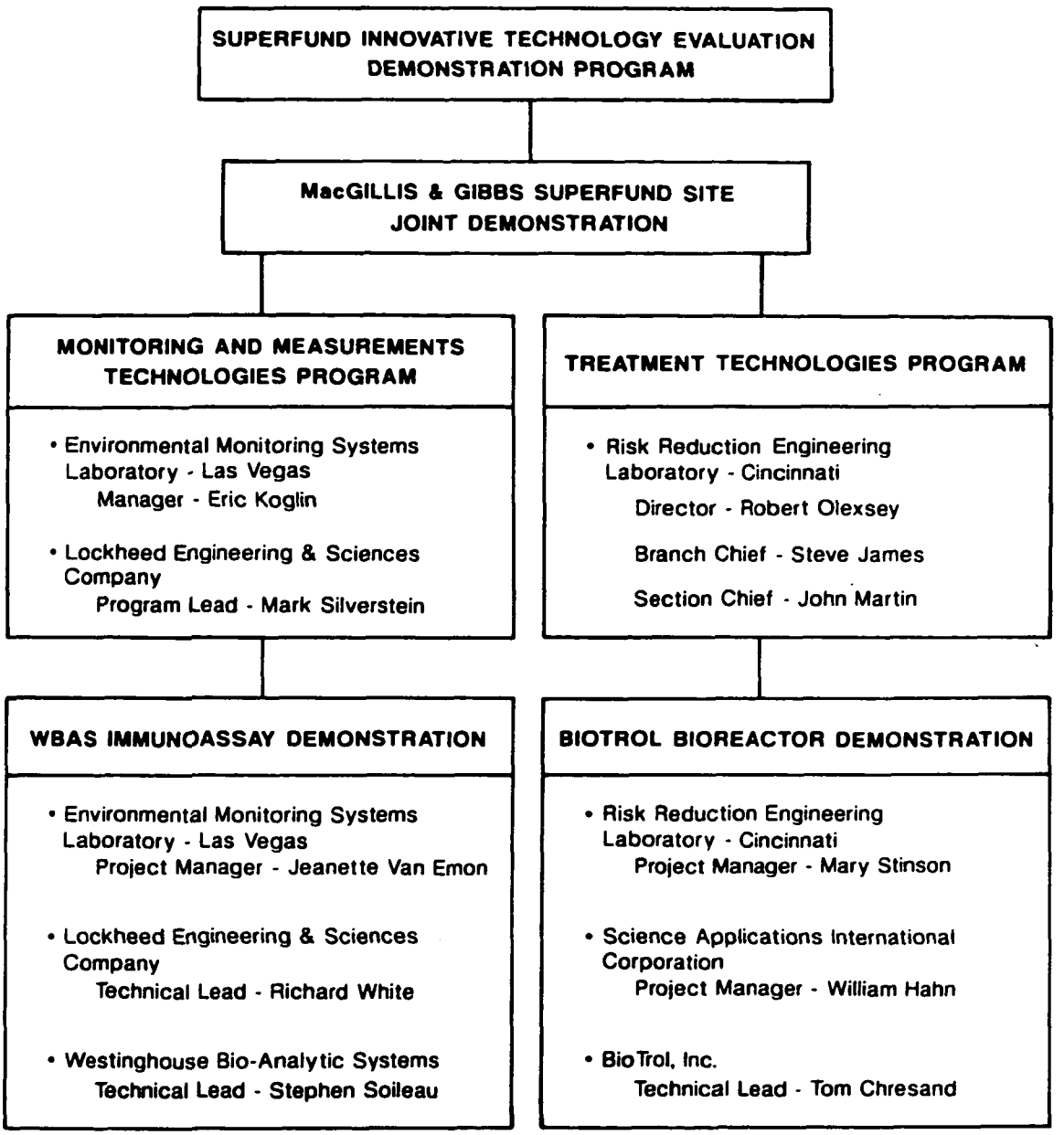


Figure 1.1. Organizational structure for the SITE Program demonstration of the WBAS pentachlorophenol immunoassays.

2. DESCRIPTION OF IMMUNOASSAY TECHNOLOGY

2.1 INTRODUCTION

Immunoassays are analytical techniques based on receptor molecules called antibodies. Quantification of the extent of contamination in an environmental sample is based on the ability of a specific antibody to bind to its target analyte. Specific antibodies can be developed to detect either a single analyte or small groups of related compounds. Laboratory-based immunoassays can accommodate an extremely high sample capacity, while portable immunoassays enable on-site analysis. Thus immunoassay techniques are quite versatile and can be applied to many analytical situations.

2.2 BACKGROUND

Immunoassay development is composed of several distinct steps: hapten design and synthesis; conjugation of hapten to carrier; immunization of animals; antibody production (either polyclonal or monoclonal); and choice of immunoassay format.

Most organic environmental contaminants are small molecules that are not large enough to stimulate the immune system to produce specific antibodies. However, when conjugated to carriers such as proteins, small molecules may elicit the production of antibodies. In these instances the small molecule is termed a hapten. Immunoassays are based on the phenomenon that the antibodies produced in response to the hapten-carrier conjugate will be able to bind to the free hapten (or target analyte) without the carrier being attached.

Antibodies are secreted by plasma cells in response to the hapten-carrier conjugates. Various immunization protocols can be used to stimulate these cells for specific antibody production. Several factors (e.g., the state of the animal's immune system, characteristics of the hapten-carrier conjugate, species response, and genetic factors) contribute to a successful immunization regime.

Polyclonal antibodies are obtained from blood serum. Within the polyclonal antiserum are antibodies of varying selectivity and sensitivity. It is only those antibodies which combine with the free hapten (or target analyte) that are useful for immunoassay development. Some of the other antibodies present may pose difficulties in configuring an immunoassay. With the aid of hybridoma technology, it is possible to select a single parent cell that produces an antibody with the desired specificity and clone it to produce millions of identical cells. These preparations are termed monoclonal, as they are a homogeneous reagent of only one antibody type. Monoclonal antibodies can be produced in large scale to provide a continuous supply of a specific antibody.

Immunoassays can be successfully developed using either polyclonal or monoclonal antibodies. Polyclonal antibodies are easier to produce and may even be superior to monoclonals in some immunoassay formats. Immunoassays based on monoclonals tend to produce steeper quantitation curves and may eliminate problems of multispecificity. Although many assay configurations are possible, each is based on the binding of a specific antibody to its target analyte(s). Assays are normally based on competition for antibody binding between a known amount of analyte and an unknown amount of analyte in a sample. An enzyme or radiolabel is bound to either the specific antibody or the analyte standards to detect and quantify the amount of unknown present. A common detection method is to determine the amount of color generated by the enzyme label and a chromogenic substrate.

Because many immunoassay formats can be used in the field, the technology can be used to provide on-site monitoring, enabling the collection of data in real time. Immunoassays are excellent methods for screening large numbers of samples. Such screening procedures would minimize the number of negative samples analyzed by expensive spectroscopic and chromatographic methods.

2.3 PCP ANTIBODIES AND IMMUNOASSAYS

Westinghouse Bio-Analytic Systems (WBAS) has developed rabbit polyclonal antiserum and rat monoclonal antibodies that are selective for PCP. Both the polyclonal antiserum and monoclonal antibody display similar selectivities towards PCP and related compounds (Table 2.1). However the polyclonal antiserum is somewhat more selective in its reactivity.

2.3.1 Field Analysis Kit Immunoassay

A polyclonal-based immunoassay field analysis kit (Figure 2.1) for detecting PCP in water has been developed by WBAS. The detection limit of this assay is approximately 3 ppb with a total analysis time of about 30 minutes. The linear dynamic range of the method is from 3 to 40 ppb. The method employs a portable spectrophotometer (Figure 2.2) for standard curve generation. The field kit provides a semiquantitative analysis of PCP in water samples. Qualitative results can also be obtained by setting the appropriate threshold values. This polyclonal immunoassay format (the field kit) will be tested under both field and laboratory conditions for the purpose of the SITE demonstration.

The field analysis kit employs an 8-well microtiter strip. Each well has a maximum capacity of 200 mL and is precoated with PCP-specific antibody by WBAS (proprietary method). Water samples are buffered and placed in the microtiter wells. A constant, known amount of enzyme-labelled PCP is then added to the well with the buffered water sample. Thus, competition between the sample and labeled PCP occurs for the antibody immobilized in each well on the strip (Figure 2.3). After a 5-minute incubation, all unbound labeled and unlabeled PCP is removed by washing. An enzyme substrate and chromogen are added, producing a colored solution. The reaction between the substrate and chromogen is stopped by charging the pH and the final colored end product is read spectrophotometrically. Figure 2.3 shows the field kit analysis steps described above. Quantification of PCP is based on the competition between PCP in the sample and the known amount of labeled PCP. The greater the amount of PCP in the water sample the lighter the color produced at the end of the assay. Conversely, if the water sample has only a

TABLE 2.1. SPECIFICITY OF ANTI-PENTACHLOROPHENOL ANTIBODIES DETERMINED BY CROSS-REACTIVITY TESTING

Compound	Molar IC_{50}[a]	Percent Cross-Reactivity with Pentachlorophenol[b]	
		Rat Monoclonal	Rabbit Polyclonal
Pentachlorophenol	$2.2 (\pm 0.3) \times 10^{-6}$	----	---
2,3,5,6-Tetrachlorophenol	$5.3 (\pm 0.6) \times 10^{-6}$	42.0	19.0
2,4,6-Trichlorophenol	$1.8 (\pm 0.3) \times 10^{-5}$	12.0	7.0
2,3,6-Trichlorophenol	$2.5 (\pm 0.1) \times 10^{-5}$	8.8	7.0
2,6-Dichlorophenol	$1.2 (\pm 0.1) \times 10^{-4}$	1.8	0.4
Tetrachlorohydroquinone	$2.8 (\pm 0.1) \times 10^{-4}$	0.8	0.7
2,3,4-Trichlorophenol	$4.5 (\pm 0.3) \times 10^{-4}$	0.5	11.0
2,3,5-Trichlorophenol	$4.3 (\pm 0.3) \times 10^{-4}$	0.5	2.5
2,4-Dichlorophenol	NI[c]	0	N/A
2,5-Dichlorophenol	NI	0	0.1
3,5-Dichlorophenol	NI	0	0.1
3,4-Dichlorophenol	NI	0	0.1
2,3-Dichlorophenol	NI	0	0.1
4-Chlorophenol	NI	0	0.2
Phenol	NI	0	0.1
Pentachloroaniline	NI	0	0.1
Pentachlorobenzene	NI	0	N/A
2,3-Dinitrotoluene	NI	0	0.1
2,4-Dinitrotoluene	NI	0	0.1
2,4,5-Trichloronitrobenzene	NI	0	0.1

[a] Molar concentration of compound that inhibits 50 percent antibody binding in immunoassays.

[b] [IC_{50} PCP/IC_{50} compound] x 100

[c] NI = Not Inhibitory; 1.0×10^{-3} M

Source: Courtesy of WBAS

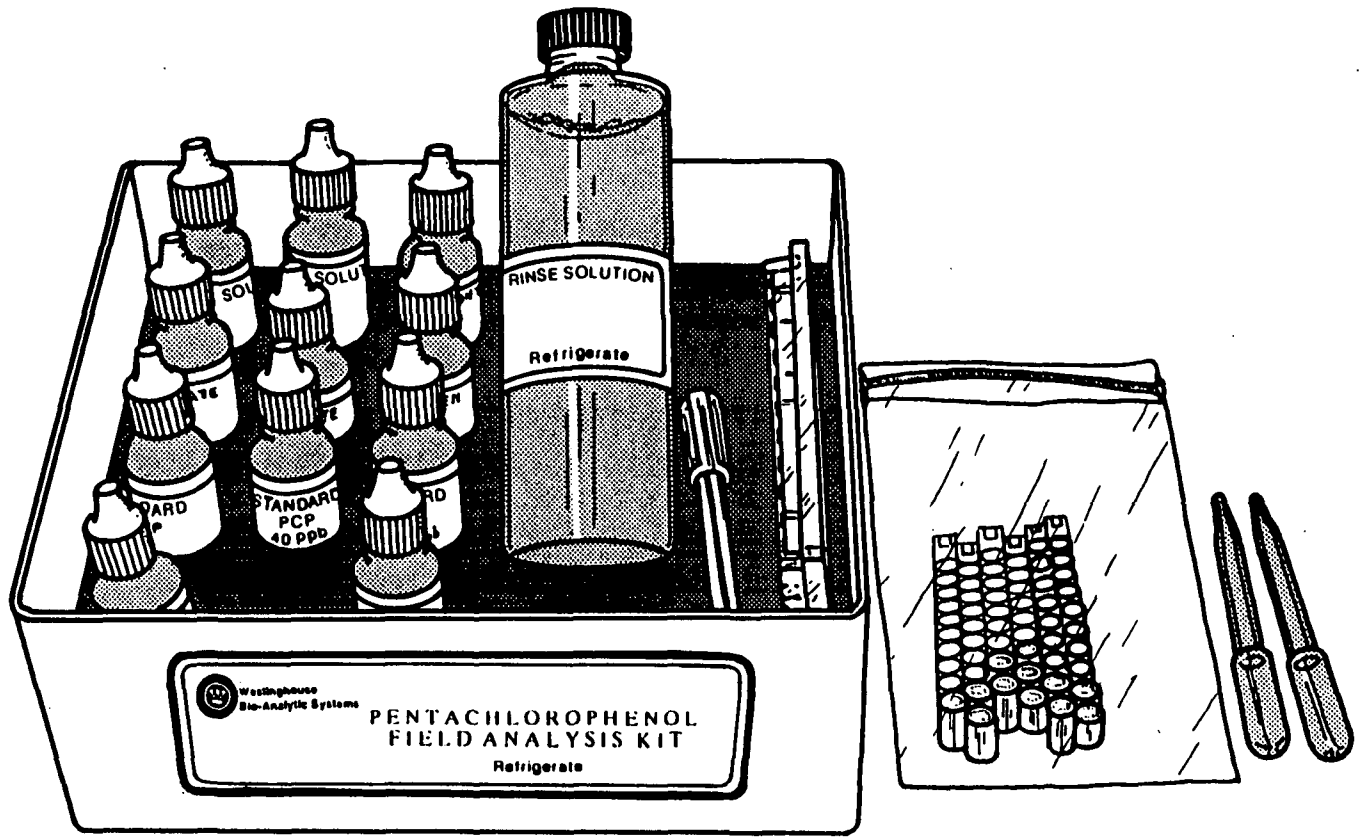


Figure 2.1. WBAS immunoassay field analysis kit for pentachlorophenol.

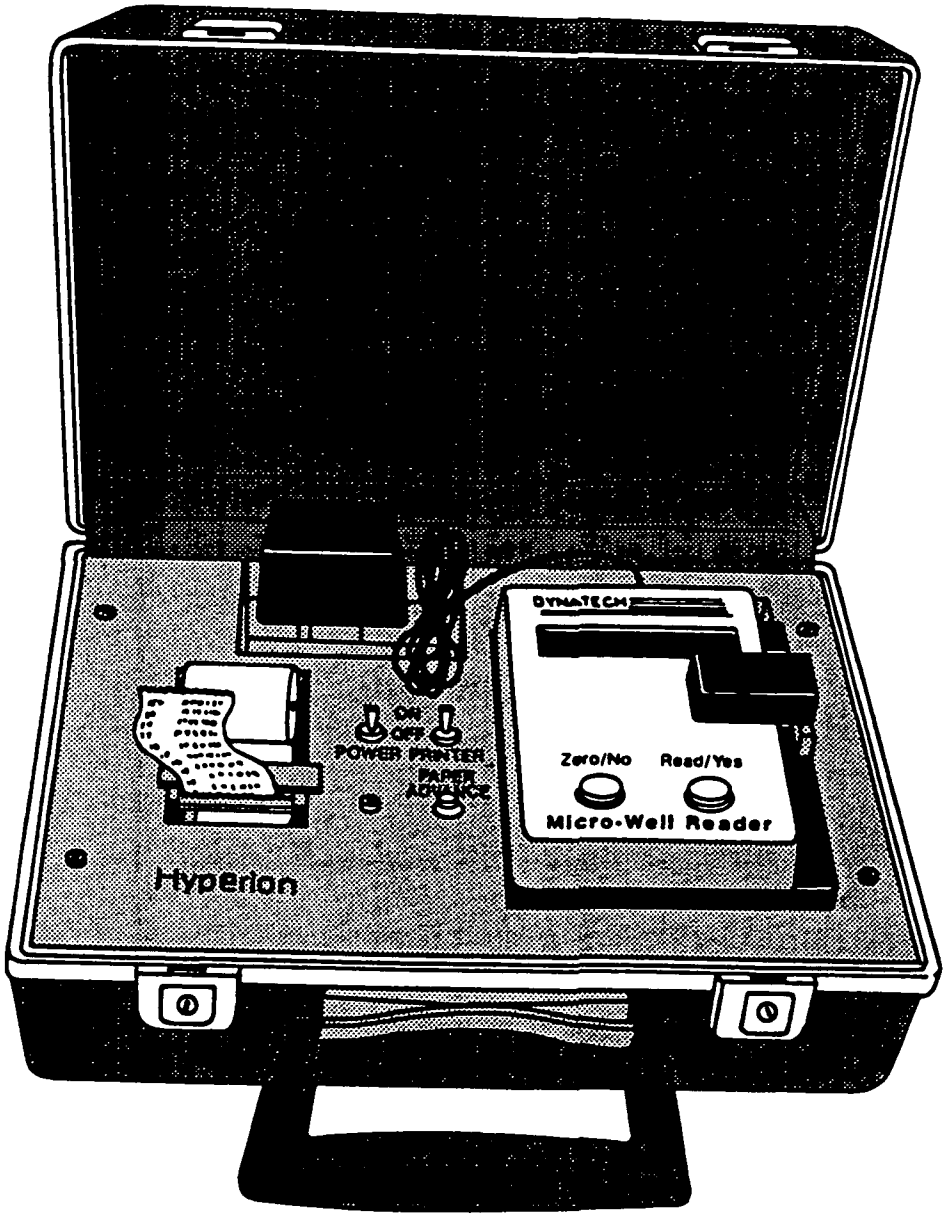


Figure 2.2. Portable microwell reader spectrophotometer.

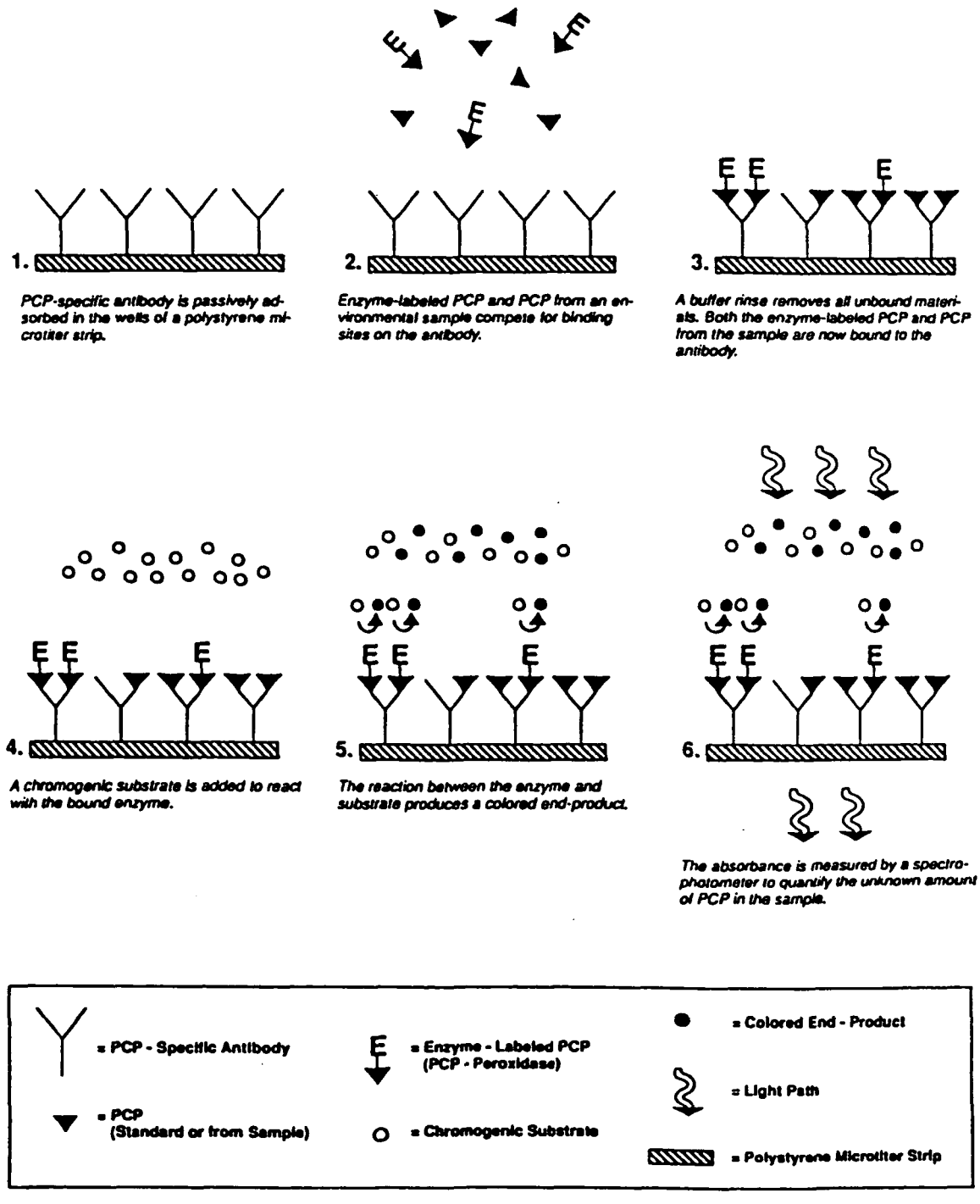


Figure 2.3 WBAS immunoassay field analysis kit procedural steps.

small amount (or no) PCP present, a dark color is produced because more of the enzyme-labeled PCP is able to bind to the immobilized antibody.

2.3.2 Plate Immunoassay

The monoclonal antibody was used to develop a quantitative immunoassay based on a 96-well microtiter plate format (see Figure 2.4 for schematics of the assay procedures). The immunoassay can detect PCP in drinking and environmental water samples. Although the immunoassay involves an overnight incubation step, it requires only 2-1/2 to 3 hours of hands-on time to complete the analysis. However, many 96-well plates can be processed at one time thus enabling a high sample capacity. Day one of the analysis is as follows: microtiter plates of polystyrene are sensitized by passive adsorption with 100 μL of a solution of the coating antigen DCP-THY (i.e., dichlorophenol conjugated with thyroglobulin) in a carbonate buffer (pH 9.6). The microtiter plates are covered with acetate sealers and stored overnight at 4 °C. Day two analysis activities include: buffering water samples with 70 mM phosphate buffer (pH 7.4) containing 1.4 M NaCl and adjusted to pH 7.4. The sample preparation is completed by diluting an aliquot of buffered sample into 25 percent by volume 2-propanol. The 25 percent 2-propanol extracts are diluted in dilution buffer containing 25 percent 2-propanol for determination of concentration range and final analysis. The dilutions typically used for range findings are undiluted, 1:10, and 1:100. Dilutions are made in 12- by 75-mm test tubes using a total volume of 1 mL and performing the liquid measurements with a Pipetteman P-1000 (Rainin Corporation). Fifty μL of prepared sample or standard is added into triplicate wells of the sensitized microtiter plate prepared on day one. Fifty μL of the anti-PCP antibody is added next, and the plate is incubated for one hour. The free PCP in the sample binds to the anti-PCP antibodies and inhibits the binding of the antibody to the solid-phase adsorbed DCP-THY conjugate. After the incubation, the anti-PCP antibodies not bound to the adsorbed PCP-THY conjugate are removed by rinsing the plate with phosphate buffer. The amount of antibody bound is indirectly determined by adding an enzyme-labeled second antibody which binds to the anti-PCP antibody. After an incubation and a final washing, the substrate of the enzyme is added, and the amount of bound enzyme is monitored by the development of a colored product. The intensity of the color is directly proportional to the amount of anti-PCP antibody bound to the solid-phase and inversely proportional to the concentration of PCP in the sample. Quantification is achieved by comparison with a standard curve.

The minimum detectable level of PCP in the plate immunoassay is estimated to be 30 ppb. When the required dilutions are taken into account, the minimum detection level of PCP in a water sample is 90 ppb. If an optional reverse-phase extraction step is used, the detection limit can be lowered to 25 ppb. If EPA Method 604 is used to extract the water samples, the minimum detectable level is below 1 ppb.

2.4 EPA EVALUATION OF THE WBAS MONOCLONAL-BASED (PLATE) IMMUNOASSAY FOR PENTACHLOROPHENOL

The monoclonal-based immunoassay (referred to in this plan as the plate immunoassay) for PCP was submitted to the EMSL-LV by WBAS for evaluation. The study (Van Emon and Gerlach, 1990) consisted of comparing the following five methods of analysis for PCP: (1) immunoassay using no extraction (i.e., direct immunoassay); (2) immunoassay detection following solid-phase

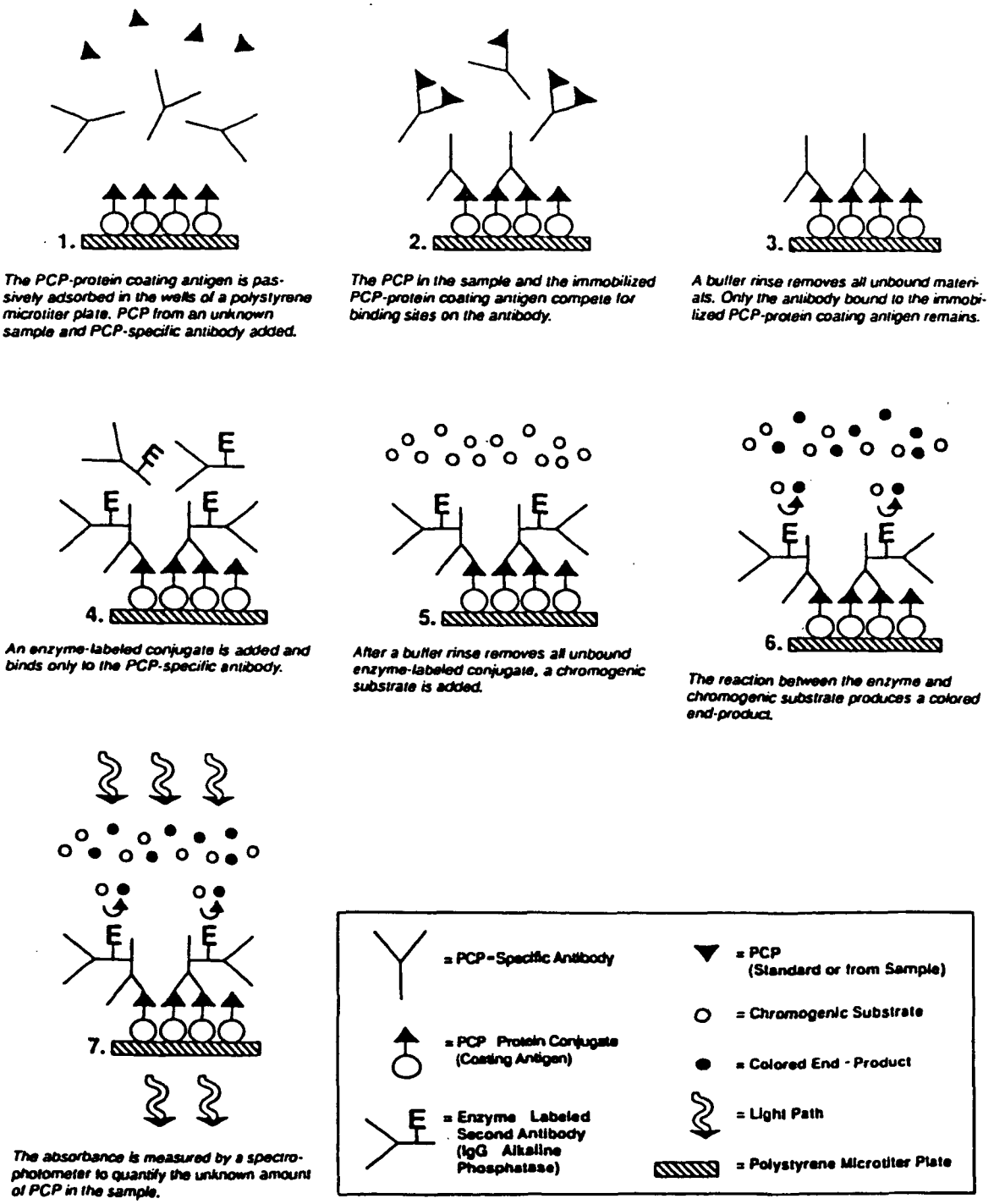


Figure 2.4. WBAS plate immunoassay procedural steps.

extraction; (3) immunoassay detection following EPA Method 604 extraction (EPA, 1979); (4) gas chromatographic (GC) detection following solid-phase extraction; and (5) GC detection (EPA Method 604 - Phenols). Samples analyzed consisted of spiked ground water, surface water, and drinking water. Each water type was collected from different geographic locations. Samples were split and spiked at two different levels of PCP. In addition, several samples were spiked with a mixture of PCP and related phenolic compounds. These additional compounds were chosen based upon the target analytes of Method 604. The inclusion of these compounds was essential to more fully evaluate the possibility of interference in the immunoassay, particularly as the immunoassay data were to be compared with those of the Agency's GC method.

Based on the conditions of this evaluation (Van Emon and Gerlach, 1990), no practical difference was observed between: (1) the plate immunoassay and GC detection of Method 604 extracts; (2) the plate immunoassay and GC detection of solid-phase extracts; (3) laboratories for PCP detection by plate immunoassay using the WBAS solid-phase or EPA Method 604 extraction protocols; and (4) precision of direct immunoassay obtained by two laboratories. As a stand-alone method the immunoassay has important applications for screening and environmental monitoring. This monoclonal-based plate immunoassay will also be evaluated in the SITE demonstration and compared with the polyclonal-based immunoassay field kit.

3. TESTING PROGRAM SCHEDULE AND DURATION

The testing program schedule and duration for the WBAS immunoassays have been designed to conform to the scheduling of the SITE demonstration of the BioTrol bioreactor.

From late May through mid-July 1989, with guidance from EMSL-LV, WBAS will conduct a variety of performance checks of the field kit in a laboratory environment. These checks will assess such operating and data quality parameters as the linear dynamic range of the calibration curve, within- and between-strip precision and accuracy, and matrix effects and interferences. A few days prior to the technology field demonstration, SAIC field personnel will conduct on-site "pilot" testing of the WBAS field kit to determine the soundness of the logistical and analytical procedures and to perform PCP concentration range checks on bioreactor system samples. These tests are detailed in the discussion of predemonstration activities (sections 4.15 and 4.16).

The WBAS field kit demonstration is planned to take place in three one-week (six-day) intervals, alternating one week on and one week off, over a period of six weeks. On or about July 24, 1989, the first set of demonstration samples is expected to be collected, analyzed on site with the WBAS field kit, and split and shipped to the off-site laboratories for confirmatory analyses. The last samples are expected to be analyzed on site on or about August 26.

4. SAMPLING AND ANALYSIS DESIGNS

4.1 TEST SITE LOCATION AND BACKGROUND

The test site for the PCP field kit immunoassay technology is the MacGillis & Gibbs Superfund Site in New Brighton, Minnesota. This is an NPL site that is well characterized with respect to the concentrations of PCP in the ground water. The EPA Fact Sheet (Appendix B) describes the site and the bioreactor demonstration in more detail and also contains a map showing the general location of the MacGillis & Gibbs site.

4.2 LOGISTICS

It is the responsibility of EMSL-LV to coordinate all logistical considerations, to ensure that all the necessary equipment and other resources are available on site, and to ensure that the proper lines of communication and the principal contacts at each on- and off-site location have been identified.

The MacGillis & Gibbs demonstration site selection and the sampling and sample shipment procedures were already in place for the BioTrol bioreactor demonstration at the onset of the planning for the field activities of the WBAS field analysis kit demonstration (Section 1.3). The bioreactor demonstration design was prepared by RREL with technical assistance provided by BioTrol, Inc.

Because RREL is responsible for implementing the sampling and analytical plans of the bioreactor SITE demonstration (SAIC, 1989), it was determined that it would be most efficient for RREL personnel to perform all the field-related activities for the WBAS field analysis kit demonstration. These activities include:

- providing sampling and shipping containers;
- collecting, splitting, and shipping samples;
- performing immunoassay field kit analysis for PCP (NOTE: WBAS will ensure that RREL personnel are adequately trained to perform the field test before field work starts);
- providing adequate space for sample analyses and equipment storage (e.g., refrigerator, mobile laboratory or other clean, safe, and sheltered work station);
- providing a health and safety plan and a plan for disposal of the solid and liquid wastes generated on site from the field kit testing; and

- providing timely access to the GC/MS analytical results for PCP determinations, as well as analytical results of the other chemical and physical measurements required for the BioTrol bioreactor demonstration.

4.3 EQUIPMENT

To ensure that all equipment is available on site in order to perform all the analyses designed into this plan, types and quantities of equipment must be anticipated, procured, and delivered to the site before field activities begin. Table 4.1 lists the equipment required for the field kit immunoassay demonstration. Table 4.2 lists the equipment required to perform the plate immunoassay.

4.4 COMMUNICATIONS

EMSL-LV will coordinate the communications network and ensure that the primary points of contact have been identified, including the names, telephone numbers, and addresses at each critical on- and off-site location. This network will ensure that sample and data flow are proper; all logistical and technical issues are quickly and properly addressed; any necessary corrective actions are addressed and approved by management; and corrective actions are documented and communicated to the principal party responsible for overseeing each operation.

4.5 PHOTOGRAPHS

A photographic log will be kept for all photographs taken to document on-site procedures and operation. This log will include date, time, subject, frame, roll number, and photographer. The photographer should review all photographs or slides for agreement with the log.

4.6 SAMPLE HANDLING

RREL personnel will collect samples to be analyzed for the WBAS field immunoassay demonstration daily (see Section 3 for scheduling) using a composite sampling apparatus (Section 4.7). These samples will be collected from two specified points in the BioTrol bioreactor (Figure 4.1): the ground-water influent (after conditioning; pH adjustment with NaOH and nutrient addition of nitrogen and phosphorus) and the treated effluent. Field equipment rinse blank water samples will also be collected from each sampling device at each sampling point (influent and effluent) on alternating days. Thus, three samples will be collected per day: one influent, one effluent, and one associated equipment wash blank from one of the two sampling points. In addition, once each week a grab sample (Section 4.7) will be collected from the influent ground water before it enters the conditioning system.

For each of the three daily samples described above, RREL field personnel will prepare split samples in amber glass, Teflon-lined, screw-cap bottles as follows:

- one (1) split bottle @ 30 mL and
- two (2) split bottles @ 250 mL

TABLE 4.1. RESOURCES AND SUPPLIES NECESSARY TO CONDUCT THE WBAS IMMUNOASSAY FIELD KIT ANALYSES FOR THE SITE DEMONSTRATION

1. Refrigerator shelf space or ice chest cooler large enough for several kits, QA standards, and samples.

2. Work space to perform test (3 ft by 3 ft) out of wind, rain, and sunlight.

3. For the shipment of samples
   a. Shipping containers, envelopes
   b. Blue ice or ice
   c. Shipping popcorn
   d. Small shipping boxes
   e. Sample bottles, amber glass, 250 mL, with Teflon-lined screw caps
   f. Bottle labels showing:
      - Sample # _____
      - Collection date _____
      - Sample type _____
      - Holding time _____
      - Name _____
   g. Shipping labels

4. For sample preparation and dilution
   a. Stock EDTA solution (1M)
   b. 10 mL disposable glass pipets
   c. Pipet bulbs
   d. Pipetman™ P-1000, P-200
   e. Disposable tips for P-1000 and P-200
   f. Disposable glass test tubes (13 mm x 100 mm) or sample vials with Teflon-lined caps for making dilutions
   g. Filters (0.2 μm), disposable syringes, 50 cc, if necessary
   h. Parafilm™
   i. Beakers and other glassware for collecting, measuring volume, and diluting samples, if necessary
   j. Test tube racks for disposable glass test tubes

(continued)

TABLE 4.1. (Continued)

5. Miscellaneous
   a. Copies of field forms or carbonless copy
   b. Pens
   c. Markers for labels
   d. Clip board
   e. Ruler
   f. Paper towels
   g. Thermometers, high and low range
   h. pH paper, hardness testing paper
   i. Hand calculator, preferably with log and statistical functions

6. Waste disposal
   a. Black cans or lined boxes for solid waste disposal
   b. Hazardous waste labels
   c. 17-C cans for liquid waste disposal, if necessary
   d. Laboratory dishwashing detergent, dishpan

7. Health and safety
   a. Disposable vinyl gloves
   b. Safety glasses
   c. Laboratory coat

8. For immunoassay analysis
   a. 6 WBAS PCP Immunoassay Field Kits per week shipped from WBAS
   b. Performance standard ampules, 25 ppm, prepared by EMSL-LV and shipped to the site
   c. A Dynatech Micro-Well Reader with power adapter and battery, Model 1 (Cat. No. 011-010-6900), equipped with a 405-nm interference filter (Cat. No. 632-800-5405)
   d. Blind or semi-blind QA audit samples prepared by EMSL-LV and shipped to the site
   e. Carboy with 10 liters of laboratory deionized water for rinsing and making dilutions
   f. Clinical table top centrifuge

TABLE 4.2. EQUIPMENT NECESSARY TO CONDUCT THE WBAS PLATE IMMUNOASSAY ANALYSES FOR THE SITE DEMONSTRATION

1. Glassware (suggested specifications)
   a. Vials: 4 to 6 mL, with Teflon-lined screw cap
   b. Volumetric pipettes: 0.1 mL; 0.25 mL; 2.5 mL
   c. Volumetric flasks: 1 mL; 5 mL; 10 mL
   d. Graduated cylinder: 2 or 4 L
   e. Hamilton syringe: 0.005 ml; 0.010 mL; 0.05 mL
   f. Powder funnel
   g. Pipetting bulbs and controllers
   h. Sidearm flask (heavy walled), 1 L

2. Plasticware
   a. Pipette tips (for single- and multichannel pipettors)
   b. Microtiter plates (96-well, polystyrene, flat bottom)
   c. Microtiter plate adhesive sealers
   d. Test tubes (polypropylene conicals with screw caps): 15 mL; 50 mL
   e. Nalgene carboy, 15 L
   f. Polyethylene wash bottles: 250 mL; 1,000 mL
   g. Reservoirs (for use with multichannel pipette), 30 mL
   h. Bottle, 1 L plastic, with screw cap

3. Pipettors
   a. Multichannel pipettor (variable volume): 5 to 50 µL
   b. Multichannel pipettor (variable volume): 50 to 200 µL
   c. Single-channel pipettor (variable volume): 1 to 20 µL
   d. Single-channel pipettor (variable volume): 20 to 200 µL
   e. Single-channel pipettor (variable volume): 200 to 1,000 µL
   f. Single-channel pipettor (variable volume): 1,000 to 5,000 µL

4. Microplate washer

5. Vacuum pump

6. pH meter

(continued)

TABLE 4.2. (Continued)

7. Refrigerator

8. Vortex mixer

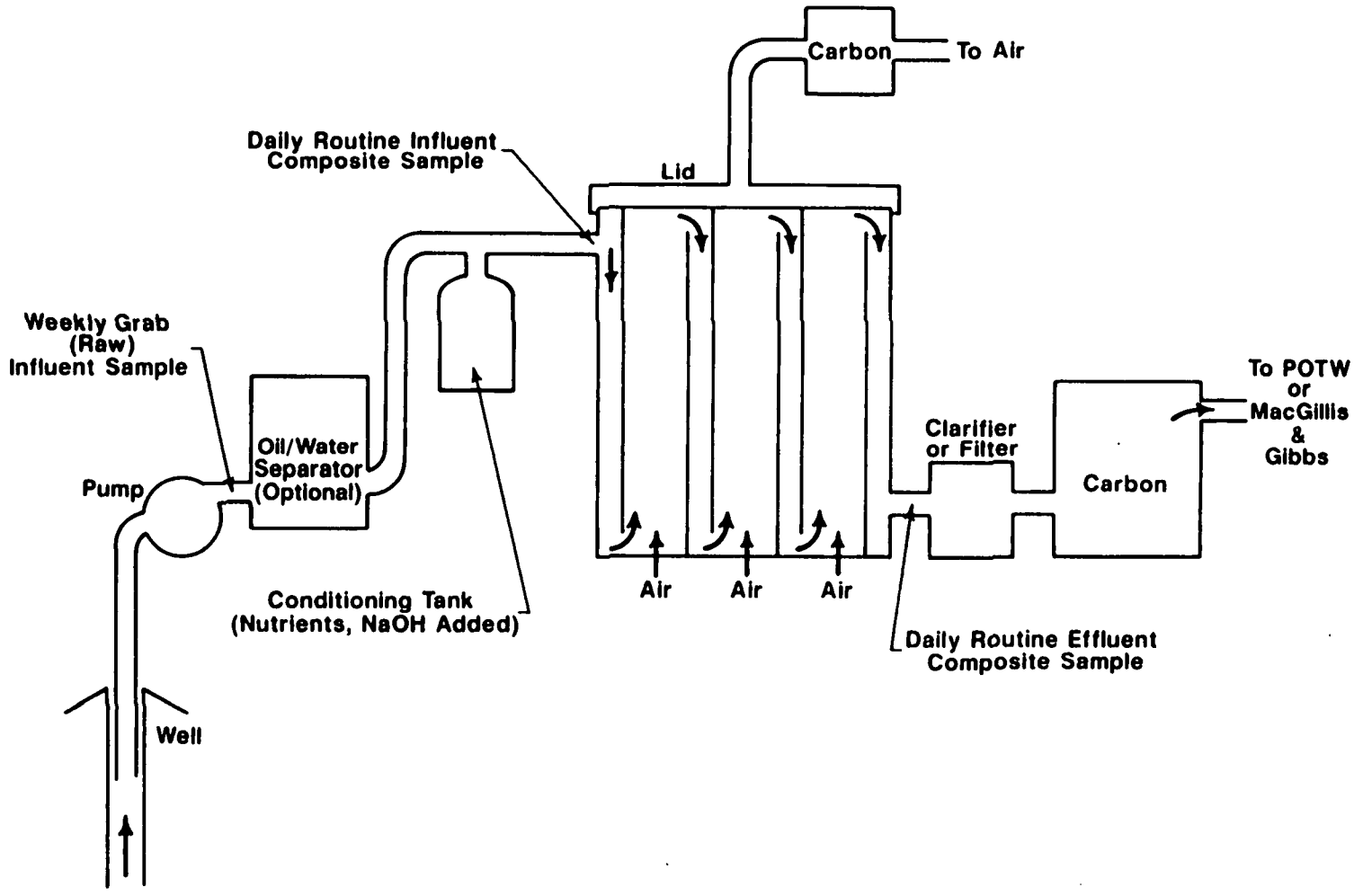
9. Magnetic stirring apparatus
   a. Stirrer with top plate large enough to hold 15 L carboy
   b. Stirring bar, 2 to 3 inches

10. Spectrophotometer: microplate reader equipped with 405 and 650 nm filters (Bio-Tek Model 310)

11. Data system[a]
   a. Compaq Computer with 640K RAM, 20 megabyte hard disk, mouse, RS232 port[b]
   b. Epson Printer - LX80[b]
   c. HP-Genechem Titercalc 2.0 software[b]
   d. Spectrophotometer/computer connection (25 pin, straight-through cable)[b]

12. Reagents
   a. Reagent grade water
   b. 2-propanol
   c. Hydrochloric acid (1 N)
   d. Sodium hydroxide solution (1 N, 10 N)
   e. Phosphate buffered saline
   f. Phosphate buffered saline-Tween 20
   g. Bovine serum albumin
   h. Microtiter plate coating antigen
   i. Monoclonal antibodies to PCP
   j. Pentachlorophenol solution in 2-propanol
   k. Developing reagent, alkaline phosphatase conjugates
   l. Diethanolamine buffer
   m. Substrate Tablets - SIGMA 104, Phosphatase Substrate Tablets
   n. Dilution buffer
   o. Sample buffer

[a] Even though it is possible to make the required calculations using a hand calculator and graph paper, this demonstration will use the listed data system components.
[b] Or equivalent component.



Source: Modified from SAIC, 1989

Figure 4.1. BioTrol, Inc., Biological Aqueous Treatment System and the sample collection points for the WBAS immunoassay demonstration.

Each 30-mL split (three per day) will be stored at 4 °C and delivered on-site to the field crew member designated to perform the field immunoassay or will be placed in the designated refrigerator for later analysis. One 250-mL split from each sample, totaling three samples per day, will be stored at 4 °C and shipped to two locations, the EMSL in Las Vegas and the WBAS laboratory in Maryland. After these samples have been analyzed according to the analysis plan (Section 4.14), they will remain in storage at 4 °C at each location until written notification from EMSL-LV specifies otherwise. On the days when the influent grab sample is collected, it will be split, shipped, and handled in the same manner as the three samples described above.

Occasionally, 1-liter splits of the influent, effluent, and grab samples, totalling about 12 over the course of the demonstration, will be prepared and sent to the EMSL-LV for GC/MS analysis. In addition, EMSL-LV will prepare and ship blind and known performance (audit) samples and a matrix spiking standard solution to the field and to the off-site laboratories (EMSL-LV, WBAS, SAIC) as specified in the analysis and QA plans (Sections 4.14 and Appendix A, respectively). Figure 4.2 provides a diagram of the sample flow described above, and Appendix D gives the procedure for sample splitting and shipment.

4.7 SAMPLE COLLECTION PROCEDURES

The demonstration plan for the BioTrol bioreactor (SAIC, 1989) describes the process of sample collection in detail and is summarized below.

The bioreactor influent and effluent samples to be analyzed using the WBAS PCP immunoassay field analysis kit will be collected with an automatic 24-hour composite sampling device. An ISCO or similar peristaltic pump sampler will be used. Each sample will be "time composited," collected in approximately 150-mL portions every 20 minutes over a period of 24 hours, for a total (i.e., bulk) sample volume of about 13 liters. The sample will be collected in a 4-gallon widemouthed composite jug located at the base of the sampler.

After the bulk (13-liter) composite sample has been collected, the 30-mL, 250-mL, and 1-L sample split bottles (specially cleaned for phenols analysis) will be prepared for on-site field analysis and for shipment to the EMSL-LV, WBAS, and SAIC laboratories.

All sample split bottles will be labelled (Figure 4.3) before splitting procedures begin. Split samples will be prepared in the same manner as the samples used for GC/MS analysis, except for the volume of sample in the split bottles. Detailed sample collection and sample split preparation procedures can be found in the bioreactor demonstration plan (SAIC, 1989).

The field equipment blank will be collected at the beginning of each composite sampling event. Blanks for the composite sampler will consist of reagent water poured into a decontaminated composite jug, pumped through the sampler, and collected directly into the appropriate bottles. Only one influent or one effluent field equipment blank will be collected and split per day.

A single influent grab sample will be collected from a garden hose attached to a T-tap located before the influent water conditioning tank. The sample will be collected directly into a bulk sample collection container similar to those used to collect the composite samples.

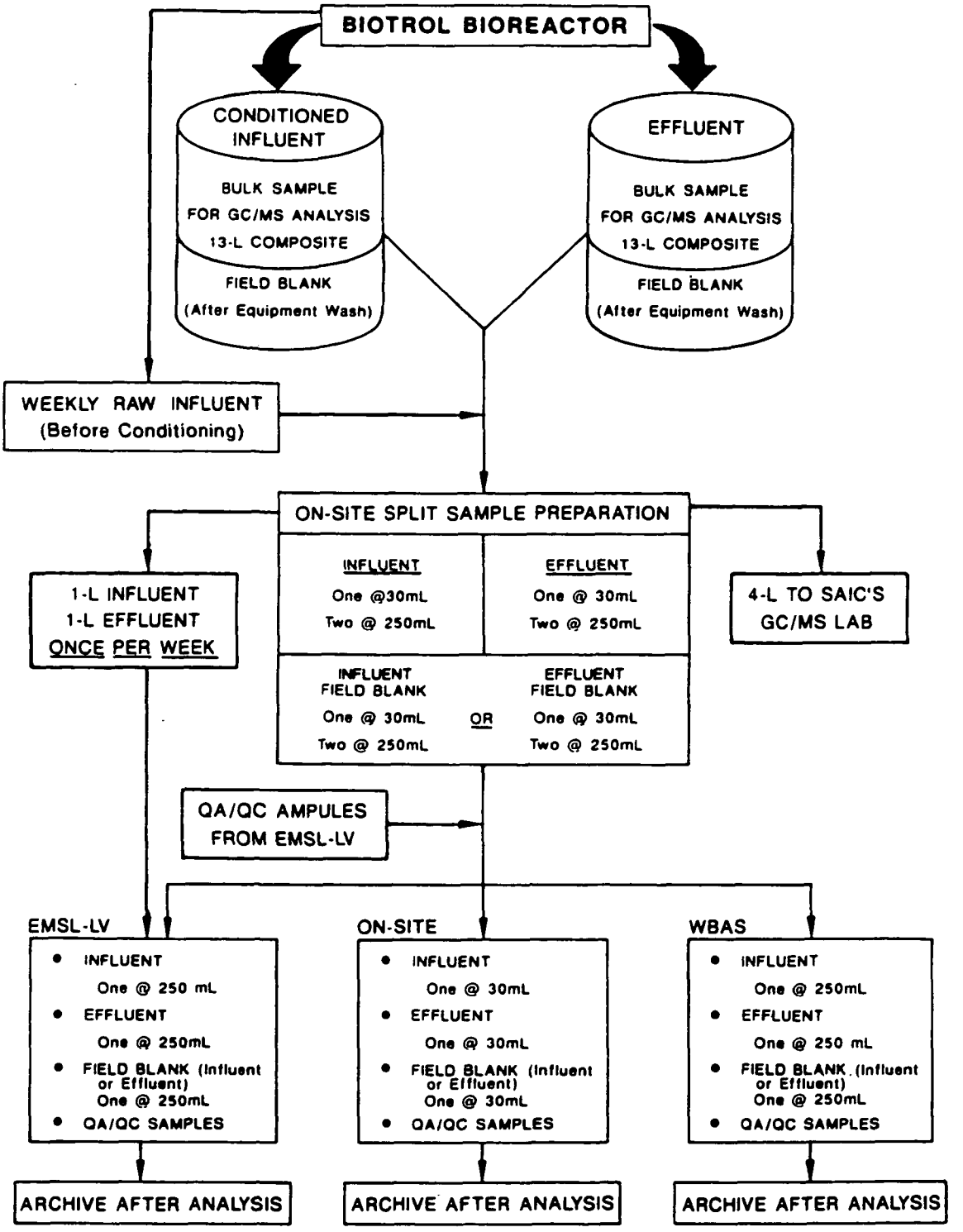


Figure 4.2. Daily sample flow (demonstration phase).

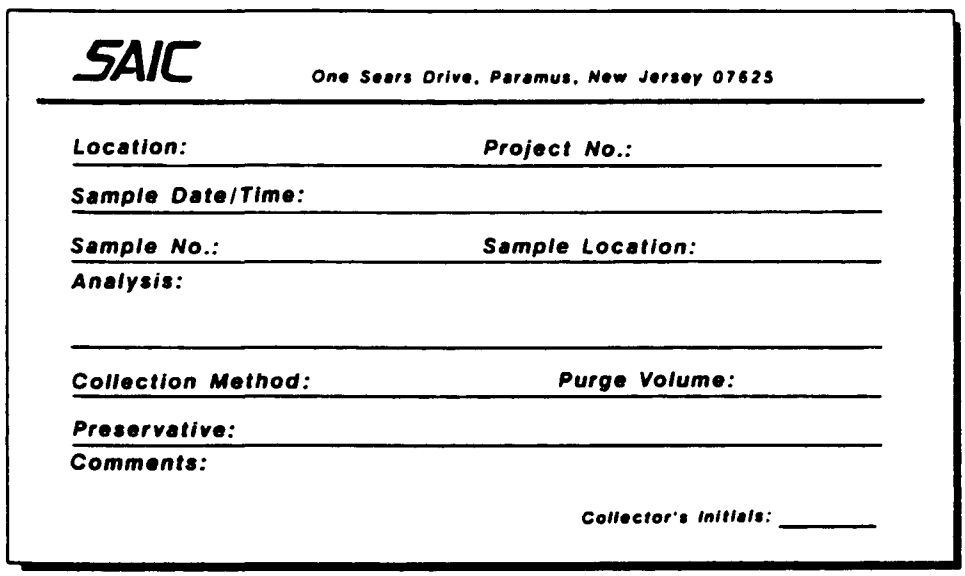


Figure 4.3. Sample bottle label.

4.8 CHAIN OF CUSTODY

Sound sample collection and analysis protocols include the implementation of chain-of-custody procedures. These procedures include sample inventory and documentation during collection, shipment (or other transfer), and laboratory processing. Chain-of-custody forms will be used to document the integrity of all samples. To maintain a record of sample collection, transfer, shipment, and receipt, a chain-of-custody form will be filled out for each sample set at each sampling location. Figure 4.4 shows the SAIC chain-of-custody form; details of the chain-of-custody procedures can be found in the bioreactor demonstration plan (SAIC, 1989).

4.9 SAMPLE PACKING AND SHIPPING

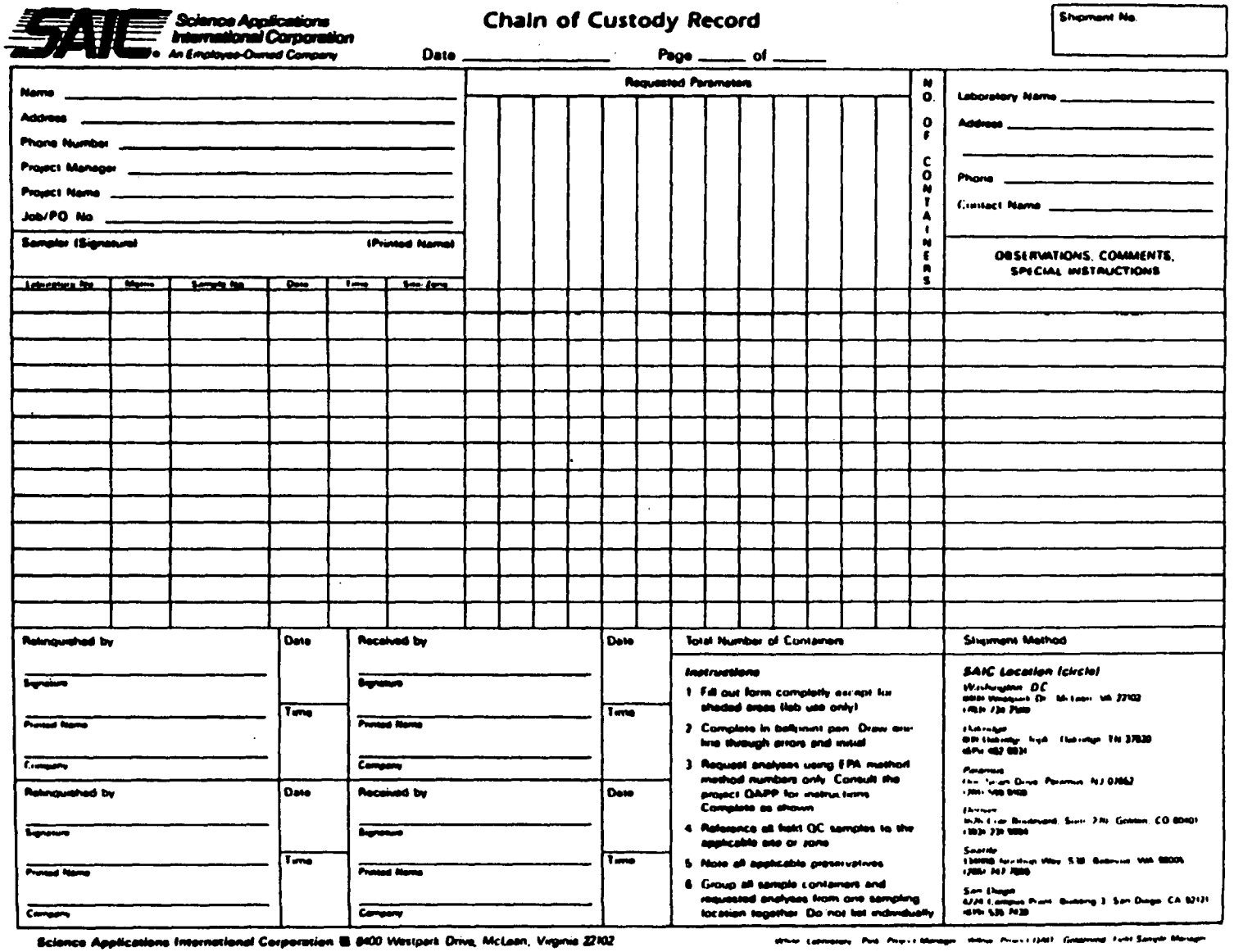
Samples will be packed with custody tape wrapped around the neck and cap of each container and then wrapped with plastic insulating material. Samples will be classified according to the Code of Federal Regulations (CFR) (BNA, 9/28/90). Sample containers will be placed in thermally insulated, rigid coolers, according to Department of Transportation (DOT) specifications. The coolers will contain ice and absorbent packing to maintain a temperature of 4 °C during shipment. The chain-of-custody form will be placed inside the shipping box. The bioreactor demonstration plan (SAIC, 1989) provides a detailed description of sample packing and shipping. NOTE: If time permits, the field data forms (Section 4.13) for the enclosed samples will be included in the box in a secured plastic bag for protection. Appendix E provides the WBAS and EMSL-LV laboratory addresses to which the split samples will be sent.

4.10 SHIPMENT OF PERFORMANCE STANDARD AMPULES

Performance standards to be used for semiblind and known QA (audit) and QC reference samples will be prepared in ampules at EMSL-LV and shipped at 4 °C via overnight courier to the MacGillis & Gibbs site and to the WBAS and SAIC laboratories in standard ampule shipping containers (30 ampules per package) (see Appendix A for use of these samples). Shipment will follow standard DOT shipping regulations for performance evaluation samples under DOT (49 CFR 173.4) exemption and in accordance with LESC shipping Standard Operating Procedure Number 20-86-40-3.

4.11 SAMPLE IDENTIFICATION

Sample tracking will be accomplished in the field by assigning each sample a unique number as it is collected. This number will be traceable back to the sample day, time, and site of collection and will also provide the tracking information for split samples analyzed on- and off-site. This sample identification number (sample ID) will be recorded on all split sample bottle labels and chain-of-custody and field data forms, as well as in a field logbook. A master log of all sample IDs will be maintained by the on-site crew chief. The sample ID system is designed to conform to the BioTrol bioreactor demonstration needs and consists of four codes representing (in order): the stage of the bioreactor demonstration ("Stage 1" or "Stage 2"); the activity period ("A," "B," or "C"); the sequential sample number collected in the activity period (01 to n + 1); and the sampling point in the bioreactor system which is coded as follows:



Science Applications International Corporation
An Employee-Owned Company

Chain of Custody Record

Date _____ Page _____ of _____

Shipment No.

Name _____
Address _____
Phone Number _____
Project Manager _____
Project Name _____
Job/PO No. _____
Sampler (Signature) (Printed Name)

Requested Parameters

NO. OF CONTAINERS

Laboratory Name _____
Address _____
Phone _____
Contact Name _____

Laboratory No.	Matrix	Sample No.	Date	Time	Sample Type

OBSERVATIONS, COMMENTS, SPECIAL INSTRUCTIONS

Relinquished by
Signature
Printed Name
Company
Date
Time
Received by
Signature
Printed Name
Company
Date
Time

Relinquished by
Signature
Printed Name
Company
Date
Time
Received by
Signature
Printed Name
Company
Date
Time

Total Number of Containers

Instructions
1 Fill out form completely except for shaded areas (lab use only)
2 Complete in ballpoint pen. Draw one line through errors and initial
3 Request analyses using EPA method method numbers only. Consult the project QAPP for instructions. Complete as shown
4 Reference all field QC samples to the applicable site or zone
5 Note all applicable preservatives
6 Group all sample containers and requested analyses from one sampling location together. Do not list individually

Shipment Method

SAIC Location (circle)
Washington DC
8400 Westport Dr. McLean VA 22102
(703) 734 7500
Knoxville
800 Oak Ridge Turnpike Oak Ridge TN 37830
(615) 482 6831
Princeton
One Tara Drive Princeton NJ 08542
(609) 452 0400
Denver
999 18th Street Boulevard, Suite 700 Golden CO 80401
(303) 239 6200
Seattle
18000 International Blvd. Suite 810 Seattle WA 98188
(206) 242 7800
San Diego
4240 Campus Point Boulevard, Building 3 San Diego CA 92121
(619) 546 7400

Science Applications International Corporation ■ 8400 Westport Drive, McLean, Virginia 22102

White: Laboratory Pink: Project Manager Yellow: Project File Goldenrod: Field Sample Manager

Figure 4.4. Chain-of-custody form.

01 - raw, unconditioned ground-water influent
02 - bioreactor conditioned influent
05 - bioreactor effluent
13 - field equipment blank

For example, the sample ID could be:

ST1-A-01-01,

which would indicate that the sample was collected at the influent point on the first day of the first activity period of Stage 1. A complete description of the sample ID process can be found in the bioreactor demonstration plan (SAIC, 1989).

4.12 SAMPLE CODING

Because of the complexity of the analysis design of the WBAS field kit demonstration, a series of sample codes has been created for the samples analyzed by the field kit immunoassay technique. These codes will identify the samples for the purpose of assessing data quality within the method and within and between analysis locations. The sample codes and their definitions are presented in Table 4.3, and a complete description of these samples can be found in Section 4.14, Analysis Design, and in the QAPjP (Appendix A).

4.13 FIELD DATA FORMS

Field data forms have been designed to document important information related to each field sample analyzed by the WBAS field kit method. These forms will be filled out on site by the field personnel analyzing samples with the field kit, as well as by laboratory personnel at EMSL-LV and WBAS who perform the field kit analysis. The field data form consists of 3-page carbonless paper; one copy will be sent from the analysis site to EMSL-LV for review and entry into a data base, one copy will be sent to WBAS for data review, and one copy will be filed on site for reference.

The form is designed to facilitate data tracking and to document analytical and field condition information. The format allows the information to be entered easily into a data base for data and statistical analyses (Section 5). Figure 4.5 shows the field form and Appendix F provides a Standard Operating Procedure (SOP) for filling out the form.

Information included on the field data form includes:

- sample collection information such as sampling date and time, sample ID, method of collection, bioreactor information, and sampling personnel;

- environmental factors such as location and date of sample analysis, meteorological conditions, sample and kit storage data, and analyst;

TABLE 4.3. WBAS IMMUNOASSAY SITE DEMONSTRATION--SAMPLE CODES AND DEFINITIONS

Quality Control Samples

FB	field equipment blank (after daily decontamination of ISCO and before daily compositing)
NC	negative control sample (1 x kit dilution buffer solution)
QC	daily quality control performance check sample at a known PCP concentration (20 ppm)

Calibration Standards

STD03	calibration kit standard at 3.0 ppb (0.0030 ppm) PCP
STD07	calibration kit standard at 7.1 ppb (0.0071 ppm) PCP
STD16	calibration kit standard at 16.9 ppb (0.0169 ppm) PCP
STD40	calibration kit standard at 40.0 ppb (0.0400 ppm) PCP

Routine Samples

RE	routine daily effluent sample
RI	routine daily influent sample
RG	routine weekly influent grab sample

Duplicates and Splits

DE	duplicate of an undiluted RE sample (i.e., split before any dilutions)
DI	duplicate of an undiluted RI sample (i.e., split before any dilutions)
SRE	split of an RE sample after the RE has been diluted into calibration range
SRI	split of an RI sample after the RI has been diluted into calibration range
SDE	split of a DE sample after the DE has been diluted into calibration range
SDI	split of a DI sample after the DI has been diluted into calibration range

Matrix Spikes

REMS-x	RE sample diluted to the range for matrix spiking which will be used in the percent recovery calculation (x = sample to be matched with the same "x" for the matrix spike effluent sample)
RIMS-x	RI sample diluted to the range for matrix spiking which will be used in the percent recovery calculation (x = sample to be matched with the same "x" for the matrix spike influent sample)
MSE-x	matrix spike sample of a REMS with corresponding "x"
MSI-x	matrix spike sample of a RIMS with corresponding "x"

Audit Samples

QAA-xxx	semi-blind QA standard, Lot A (xxx = LESC sample control number)
QAB-xxx	semi-blind QA standard, Lot B (xxx = LESC sample control number)

Cross-Calibration Standards

CC-xxx	cross-calibration color check standard for spectrophotometers (xxx = code for theoretical optical density)

FIELD DATA FOR ANALYSIS OF WBAS FIELD IMMUNOASSAY TEST KIT

Sample Collection Information

Sample Date: _ _ _ _ _ _ _ O Sample Time: _ _:_ _ O SAIC Sample No.: _ _ _ - _ - _ _ - _ _ O
Collection Method: Composite/Grab ISCO ID: ________ O Preservative: ___________ O
Bioreactor ID:____ Groundwater Well No.:____ Flow Rate: __ gpm;
BATS PCP Spike: Y/N; BATS Spike Conc: __ ppm
Collected by: ______ Split by: ______
Comments:

Environmental Factors

Analysis Date: _ _ _ _ _ _ _ O Days Since Collected: _ _ O Site of Analysis: On-Site/WBAS/LV O
Location of Analysis: Indoors/Outside Location Temp: _ _ °C
If Outside, Sunny/Cloudy/Mixed O Windy/Calm O Other ___________ O
Kit Lot No.:_________ O Kit Storage: Days @ Ambient Temp:_ _ O; Days @ 4°C: _ _ O
Daily Refrig. Temp: _ _ °C O
Comments:

Analyst: _______

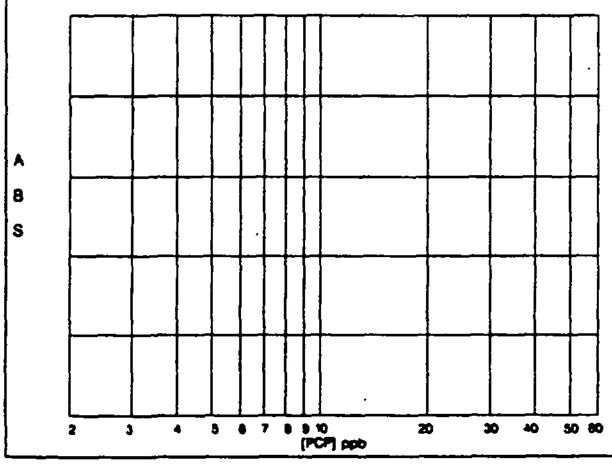
Sample-Specific Pre-Analysis Information

Sampling Point Source: Influent/Effluent/In-Reactor(_)/Other _____ O
Sample Appearance O: Color: _____ O; Clear/Turbid O; Precipitate: Y/N O; Oil: Y/N O; Other: _______ O
Centrifuged: Y/N O If yes, Appearance Change: Y/N
If yes, Color:_____ O; Clear/Turbid O; Precipitate: Y/N O; Oil: Y/N O; Other: _______ O
pH-Meter: _ _._ _ O pH-Paper: _ _._ O Hardness-Paper: _ _ _ O
Field Blank: Y/N SAIC Sample No.: _ _ _ - _ - _ _ - _ _ O
Field Duplicate: Y/N SAIC Sample No.: _ _ _ - _ - _ _ - _ _ O
Field QA Audit: Y/N LESC Sample No.: _ _ _ - _ _ _ O
Comments:

SAMPLE ANALYSIS INFORMATION BY STRIP

Strip ID: _ - _

Sample Code	Well #	OD (ABS)	Plotted Conc (ppb)		to ppm		Dilution Factor		Sample Conc (ppm)	Amt(ppb) Spike Added	% Spike Rec
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—
______ O	—	—.—	—.—	X	0.001	X	____	=	—.—	—.—	—.—



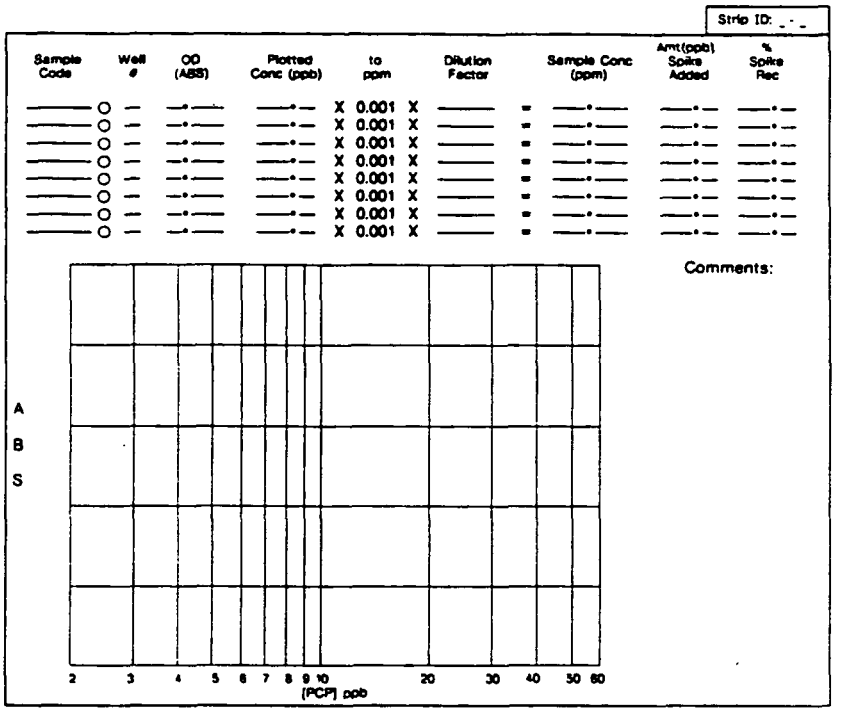
Comments:

Figure 4.5. Field data form (page 1 of 2).

FIELD DATA FOR ANALYSIS OF WBAS FIELD IMMUNOASSAY TEST KIT

SAIC SAMPLE NO.: _ _ _ - _ - _ _ - _ _ ANALYSIS DATE: _ _ _ _ _ _ _ ANALYST: _____

SAMPLE ANALYSIS INFORMATION BY STRIP



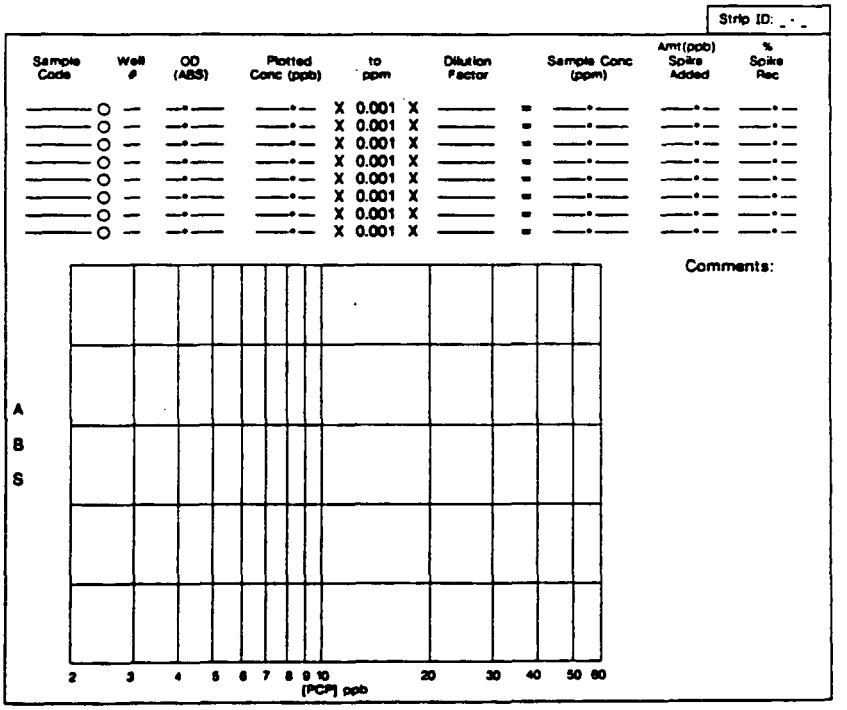


Figure 4.5. Field data form, (page 2 of 2).

- sample information before kit analysis such as sample appearance (before and after centrifugation, if applicable), pH, and sample tracking data on field QA (blank, duplicate, audit) samples; and

- kit sample analysis information such as sample codes, sample optical densities, semilogarithmic graph paper for plotting absorbance units, and a method to determine sample concentrations and matrix spike percent recoveries.

4.14 ANALYSIS DESIGN

The SITE demonstration plan for the BioTrol bioreactor (SAIC, 1989) contains detailed information regarding sample collection, handling, storage, and the analysis of samples for PCP (and PAH) by GC/MS and other analytical test methods (see Appendix C for a list of the other analytes to be determined on the bioreactor water samples).

The sampling points in the bioreactor system chosen for sampling and analysis in the field kit demonstration were selected because these are the first and the last points in the bioreactor process that will be collected on a daily basis and analyzed for PCP by GC/MS. The state of the water samples at these two sampling points in the bioreactor system will include: (1) groundwater that has been conditioned by adding NaOH to adjust the pH to ~7.3 and nutrients (i.e., nitrogen and phosphorus compounds) to sustain the microbes in the bioreactor and (2) effluent water from the bioreactor before the removal of solids and before carbon filtration. Field duplicate samples of the influent and effluent samples described above will be collected periodically with a second composite sampler located beside the first. Samples of field equipment blank water rinsate (field blank) samples will also be collected after daily decontamination of the composite sampler. In addition, once per week an influent grab sample will be collected from the bioreactor system at a point before the water is conditioned.

The analysis scheme and schedule for each operating day and schematics for the daily analyses are presented in Appendix G. The specific standard operating procedure (SOP) for performing analyses with the field kit, including sample dilution instructions and QC criteria, is found in Appendix II of the QAPjP (Appendix A).

Split samples will be analyzed by using the immunoassay field kit on site and at the EMSL-LV and WBAS laboratories. Splits of the same samples will also be analyzed by the quantitative plate immunoassay method at EMSL-LV and WBAS. These analyses will be compared to the GC/MS analyses for these splits performed at the SAIC laboratory in San Diego, California (with a select group of samples also to be analyzed by GC/MS at the EMSL-LV facility).

WBAS has also provided SOPs for the plate immunoassay and for the cleanup of samples on solid phase extraction columns, if it is necessary to use that technique (based on the matrix spike recovery criteria of 100% ± 25%). The SOP for sample cleanup contains detailed instructions for sample preparation and column conditioning loading and elution. The average expected recovery for the columns (~86 percent) was determined through studies with ^{14}C-labelled PCP. The document entitled "Quality Assurance Plan for Immunoassay Evaluation and Research Projects" (White and Miah, 1989) contains information on protocols for instrument and plate variability checks and other QA and QC requirements relevant to immunoassays.

All SOPs for the field activities related to the WBAS field kit test will be provided to on-site personnel during the set-up activities. Additional information will be supplied including instructions for sample splitting, labelling, storage, and shipment; the schedule and instructions for performing the PCP field kit analysis; instructions for handling and analyzing the QA and QC samples (Appendix H); instructions for documenting field data (Appendix F); and health and safety information (Section 6).

Samples will be analyzed following EPA Method 8270 (Gas Chromatography/Mass Spectrometry for Semi-Volatile Organics; EPA, 1986). This method contains detailed analysis instructions, QC guidelines, and performance data for PCP analysis by GC/MS. In addition, EPA Method 3510 (U.S. EPA, 1986) will be used for sample extraction.

Table 4.4 presents a summary of the expected performances of each of the three analytical methods for PCP analysis used in this demonstration. Figure 4.6 shows the relationship of the three methods as well as the location of the analysis of each. Section 5.3 provides an overview of the ways in which the three methodologies will be compared.

4.15 PREDEMONSTRATION PLAN DESIGN AND STRATEGY

The following activities will be included in the preliminary evaluation phase of the WBAS PCP immunoassay demonstration:

1. SOPs for the field activities (sample handling, analysis, and shipment) will be provided to field personnel. In addition, a field form with detailed instructions and sample codes and instructions for QA/QC sample analysis will be provided.

2. Drafts of the Immunoassay SITE Demonstration Plan and QAPjP will be disseminated to the appropriate parties for concurrence.

3. EMSL-LV, WBAS, and SAIC personnel will cross-calibrate the microwell strip spectrophotometer at the three analysis locations and with the laboratory spectrophotometers at EMSL-LV and WBAS on test standard (N-2,4-DNP glycine) curves.

4. WBAS will prepare and ship immunoassay reagents for the field kit PCP methods to the field site (as facilities become available) and to EMSL-LV. The quantities will be sufficient for one week of analysis. Subsequent reagent shipments will be made on a weekly basis during the demonstration activities.

5. A list of supplies and equipment required at the field site will be circulated to the parties responsible for procurement (WBAS, LESC, or SAIC). These supplies will be shipped to the field site as soon as the personnel and facilities (e.g., refrigerator) are in place.

TABLE 4.4. KNOWN OR ANTICIPATED PERFORMANCES FOR PCP ANALYSIS

Performance parameters	Field analysis kit	Lab plate immunoassay	GC/MS (EPA methods 8270, 3510
Detection limit (ppb)	3	~ 10-15	~ 30-50
Linear dynamic range (ppb)	3-40	~ 30-400	30-200
Precision	10-15% RSD	5-10% RSD	[a]
Accuracy	NA[b]	NA	[c]
Time to analyze	~30 minutes	~2.5 hours	~1 hour
Cost per sample	5 samples per analysis ~$30 per sample	10 samples per analysis ~$25 per sample	~$700
Key interferents	See Table 2.1	See Table 2.1	No major
% Matrix spike recovery	50-150%	75-125%	14-176%[d]
Fieldability (on site/mobile laboratory)	Yes	Yes	No

[a] Robertson (1989) reports within-laboratory precision of 21% RSD; between laboratory precision of 36% RSD based on approximately 50 laboratories.

[b] NA = not available.

[c] Robertson (1989) reports matrix spike/matrix spike duplicate accuracy of 15.5 percent based on approximately 50 laboratories.

[d] Robertson (1989) reports matrix spike recoveries of 71 and 79 percent based on approximately 50 laboratories.

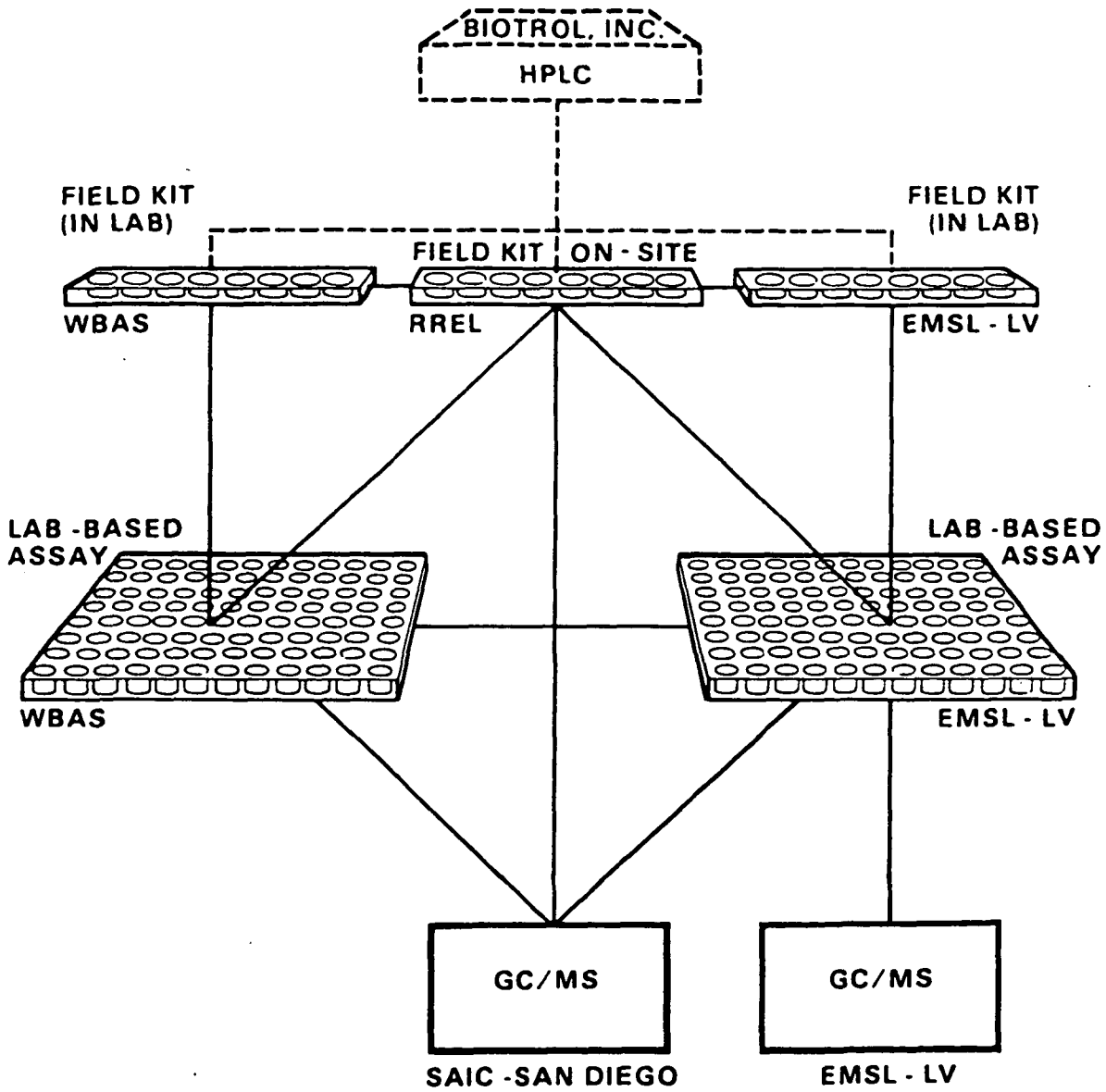


Figure 4.6. Comparison scheme of pentachlorophenol analysis methods employed in the WBAS immunoassay field kit demonstration.

6. EMSL-LV will prepare QA and QC samples for both (field kit and plate) immunoassay formats and for the GC/MS method. Performance materials obtained from EMSL-Cincinnati will be diluted in methanol and ampulated. EMSL-LV will ship the ampules, along with SOPs for handling and analysis, to each location performing the analyses.

7. Biotrol, Inc., will send WBAS and EMSL-LV samples of PCP-contaminated well water from the MacGillis & Gibbs site at various concentrations; surface water with low-level PCP contamination (>0.5 ppm); and archived and on-line bioreactor influent, effluent, and conditioning tank water. These samples will be analyzed by WBAS and EMSL-LV for preliminary matrix testing to determine if any sample pretreatment measures will be necessary during the demonstration.

8. WBAS will provide EMSL-LV with data on accuracy, precision, detection, possible interferences, linear dynamic range, and other performance characteristics of the field PCP immunoassay method.

4.16 PREDEMONSTRATION ON-SITE ANALYSIS

The purpose of conducting an on-site preliminary test of the field kit is to (1) perform range-finding and interference checks on influent and effluent samples and (2) evaluate the efficiency of the analysis and logistical systems, including communications and data flow and documentation.

If possible during the predemonstration testing phase, a few influent, effluent, blank, and QA/QC samples will be analyzed on-site. Split samples will be shipped to WBAS and EMSL-LV for analysis by the field kit and plate immunoassay methods. Field forms will be filled out and analytical SOPs will be followed to confirm proper understanding of the requirements.

5.0 DATA ANALYSIS AND MANAGEMENT

5.1 DATA HANDLING

Data generated in the field will be collected and documented on the field data forms. One copy of each form will be sent to the data base manager at EMSL-LV, one will be sent to WBAS, and one will be retained on site. Data generated from the field kit method performed in the EMSL-LV and WBAS laboratories will also be recorded on the same field data forms used on-site; a copy will be sent to the data base manager and a copy will remain at the point of analysis. Data generated from the plate immunoassay by EMSL-LV and WBAS will be sent to the data base manager on hard copies and on a floppy diskette if necessary. Copies of the data generated from the GC/MS analysis at the SAIC laboratory will be sent on data reporting and documentation forms used by SAIC. Figure 5.1 provides a diagram of the data flow.

All locations will be contacted by EMSL-LV for preliminary data over the telephone until the data forms or diskettes are received. All data will be reviewed by a QA officer for consistency in reporting, reasonableness, transcription errors, other data reporting errors, and other issues. After review the data will be entered into a data base (Section 5.2), from which a detailed statistical review will be performed.

5.2 OVERVIEW OF DATA BASE DESIGN AND DATA BASE MANAGEMENT

The data collected from the SITE immunoassay demonstration will be entered and stored in a Statistical Analysis System (SAS) data set. The SAS data set will be divided into separate files (members), depending on the source of the data (RREL, EMSL-LV, SAIC, WBAS) and on the analytical method (field kit immunoassay, plate immunoassay, GC/MS). (NOTE: BioTrol, Inc., has developed a quick turn-around high-pressure liquid chromatography method for analyzing PCP that is not part of this demonstration. However, any data generated analyzing samples with this method may be useful for additional comparisons.)

Variable names that identify samples will be complete and consistent across members to allow for both within-method performance assessment and between-method comparisons as the need arises. Each member will contain variables for all information pertinent to that analysis.

5.3 OVERVIEW OF DATA ANALYSIS

The data analysis will consist of two phases. In the first phase the quality of the data will be assessed. This assessment will consist of a number of standard procedures. The QA and QC data will be examined (via time trends and descriptive statistics) to ensure that consistent results and adequately accurate measurements are available. This examination will require data from the various blanks, audit samples, matrix spikes (Appendix J), and splits and duplicates. This form of

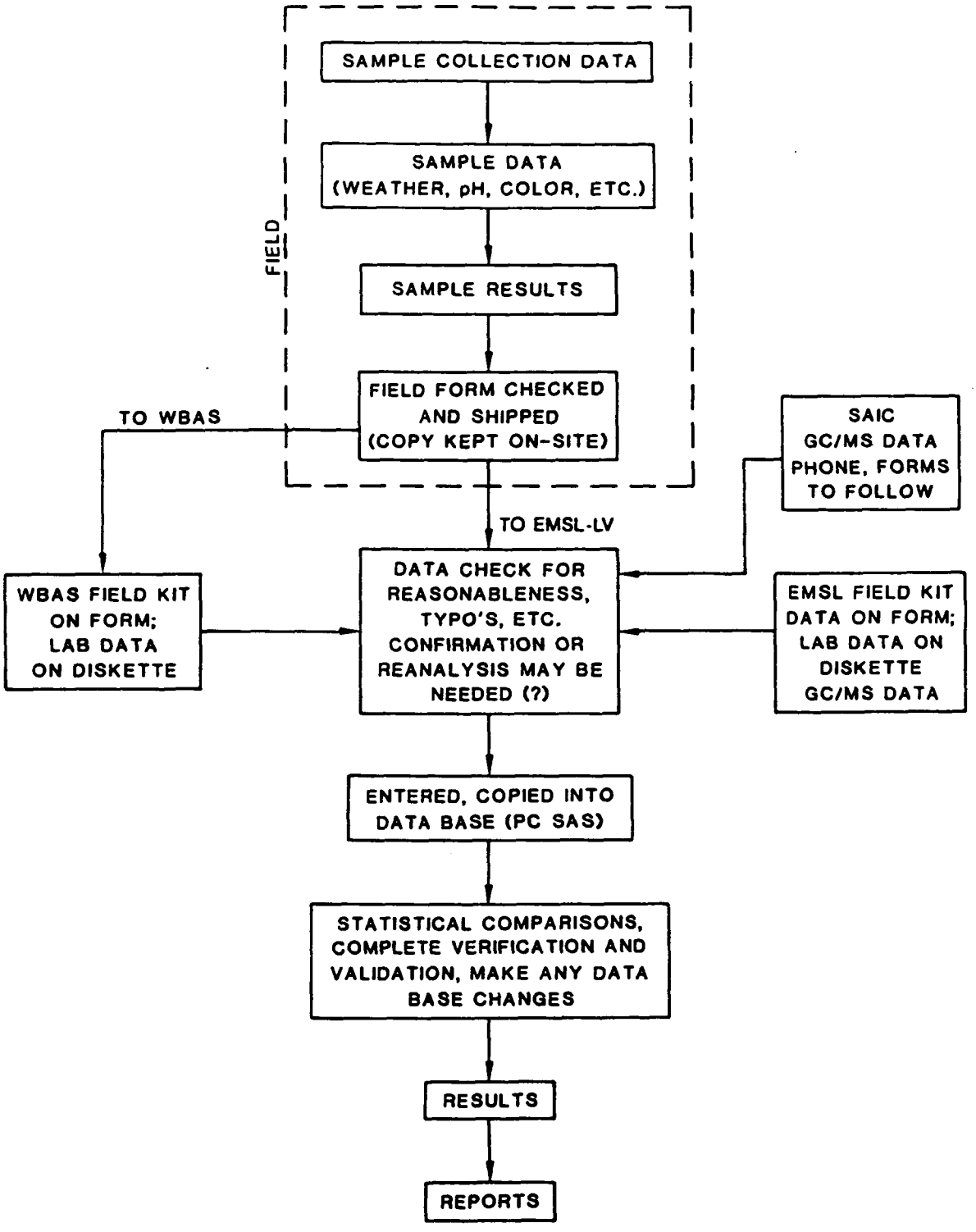


Figure 5.1. Data flow (demonstration phase).

analysis will be conducted for each analysis method for each site. Also, based upon the results of the splits and duplicate analyses between and within strips, a simple analysis of variance (ANOVA) to determine relative sources of error may be carried out if it appears, from inspection of the data, that a major component of error can be isolated. The second phase of analysis will be the comparison between methods and sites. Of primary concern will be the comparison between routine GC/MS values and the field kit results. Given positive results from the first phase of analysis, it will be assumed that the GC/MS results are the "true" values with respect to the accuracy expected from the immunoassay method. The comparison will be examined via summary statistics, t-tests for differences, correlation of results, and XY plots. Similar analyses are planned for comparing results from the other tests, though these latter results are expected to provide less important information because the primary goal is to look at the strip immunoassay under field conditions. However, efforts will also be made to estimate whether laboratory bias is significant as well as whether method-to-method bias exists.

6. HEALTH AND SAFETY

Most of the on-site health and safety considerations are described in the bioreactor demonstration plan (SAIC, 1989). The following instructions relate to the analysis aspects of the WBAS PCP immunoassays:

1. Because PCP is both a toxic and carcinogenic substance (see Appendix I, Materials Safety Data Sheets), solutions containing the substance should be handled with care. Personnel handling PCP-containing solutions will wear plastic or vinyl gloves, laboratory coat, and safety glasses. Care should be taken to avoid skin exposure, since the substance can be absorbed through the skin.

2. The QA and QC solutions contain methanol, an inflammable solvent, in addition to parts per million concentrations of PCP. The ampules have a warning label and will be shipped according to DOT regulations. These solutions should be handled with care, as they are flammable and toxic.

3. For waste disposal of liquids contaminated with PCP, use empty glass solvent bottles, 17-C cans, or 55-gallon drums. Make sure the containers are labelled with hazardous waste labels and that a record is made showing the solvent type and the approximate volume and concentration of PCP.

4. Small pieces of PCP-contaminated solid waste, such as disposable Pasteur pipets and pipet tips, should be placed in empty glass solvent bottles or in a plastic trash bag inside of a metal can or thick-walled cardboard box. These containers should be labelled with hazardous waste labels stating that the contaminant is PCP.

5. Reusable glassware contaminated with PCP should be rinsed with acetone or methanol prior to placing it with other glassware for dishwashing. The solvent rinses should be collected and disposed of with other PCP waste.

6. The colorimetric substrate used in the WBAS field kit is carcinogenic and should be handled with the same precautions applicable to the solutions containing PCP (see above).

REFERENCES

BNA (Bureau of National Affairs, Inc.). September 28, 1990. Chemical Regulation Reporter. 49 CFR 173.4. Exceptions for Small Quantities, Bureau of National Affairs, Inc., Washington, D.C.

EPA (U.S. Environmental Protection Agency). 1979. Method 604-Phenols. Federal Register 44:69464-69575.

EPA (U.S. Environmental Protection Agency). 1986. Test Methods for Evaluating Solid Waste: Method 3510; Method 8270. EPA/SW-846. U.S. EPA Office of Solid Waste and Emergency Response, Washington, D.C.

Robertson, G. L. 1989. A Dynamic Method Validation Report on the Contract Laboratory Program Routine Organic Analysis. Internal Report. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada.

SAIC (Science Applications International Corporation). 1989. Draft Demonstration Test Plan for BioTrol, Inc. Biological Treatment of Contaminated Groundwater at New Brighton, Minnesota. Draft Document. Science Applications International Corporation, 1 Sears Drive, Paramus, New Jersey.

Twin Cities Testing Corporation. 1986. Remedial Investigation Report MacGillis and Gibbs Company Site Prepared for Minnesota Pollution Control Agency. #120 86-414.

Van Emon, Jeanette M., and Robert W. Gerlach. 1990. EPA Evaluation of the Westinghouse Bio-Analytic Systems Pentachlorophenol Immunoassays. EPA/600/X-90/146. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada.

White, R. J., and M. J. Miah. 1989. Quality Assurance Plan for Immunoassay Evaluation and Research Projects. Internal Document. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada.

APPENDIX A

QUALITY ASSURANCE PROJECT PLAN FOR THE SITE DEMONSTRATION OF THE WESTINGHOUSE BIO-ANALYTIC SYSTEMS IMMUNOASSAYS FOR PENTACHLOROPHENOL

The following Quality Assurance Project Plan (QAPjP) was approved in July 1989 and was used as guidance for the demonstration field activities.

QUALITY ASSURANCE PROJECT PLAN FOR THE SITE DEMONSTRATION OF THE WESTINGHOUSE BIO-ANALYTIC SYSTEMS IMMUNOASSAYS FOR PENTACHLOROPHENOL

Prepared by:

Lockheed Engineering & Science Company
1050 East Flamingo Road
Las Vegas, Nevada 89117

Prepared for:
United States Environmental Protection Agency
Environmental Monitoring Systems Laboratory
Las Vegas, Nevada 89193-3478

PROJECT QUALITY ASSURANCE PLAN APPROVAL

This plan is approved for use with the SITE Demonstration of the Westinghouse Bio-Analytic Systems Immunoassay for Pentachlorophenol (PCP).

This project quality assurance (QA) plan was developed to assure that all environmental data generated for the U.S. Environmental Protection Agency (EPA) are scientifically valid, representative, comparable, complete, and of known accuracy. The signatures of key project personnel below indicate concurrence with the procedures specified in the plan and a commitment to disseminate the plan and the philosophy of quality to all project personnel.

V. Eckers, QA Officer, LESC — Date 8/11/89

J. Van Emon, Project Manager, EAD — Date 8/14/89

R.J. White, Principal Scientist — Date 8/11/89

E. Koglin, Manager, QAD — Date 8/15/89

W.D. Munslow, Manager, LESC — Date 8/11/89

D.G. Easterly, QA Officer, EMSL-LV — Date 8/15/89

CONTENTS

QUALITY ASSURANCE PROJECT PLAN FOR THE SITE DEMONSTRATION OF THE WESTINGHOUSE BIO-ANALYTIC SYSTEMS IMMUNOASSAYS FOR PENTACHLOROPHENOL

INTRODUCTION:

One of the goals of the U.S. Environmental Protection Agency's Superfund Innovative Technology Evaluation (SITE) Program is to evaluate the application of new and innovative technologies for field measurement of environmental contaminants. In this SITE demonstration, the technology being evaluated is a rapid field-deployable immunoassay, which is specific for pentachlorophenol (PCP). The analytical method employs an antibody which specifically binds to the target analyte PCP. In this SITE demonstration, the immunoassay will be evaluated as a rapid, semi-quantitative field screening method.

To ensure that the data resulting from this SITE demonstration are of sufficiently high quality to meet the intended use, it is necessary to prepare a Category II Quality Assurance Project Plan. The following QA Project Plan addresses the eleven key elements that are necessary for Category II projects (U.S. Environmental Protection Agency, "Quality Assurance Program Plan." EPA/600/X-87/241. Environmental Protection Agency. Las Vegas, Nevada, 1987.)

STARTING DATE:

Preliminary evaluation phase: June 26 to July 21, 1989
Formal data collection: July 24 to August 26, 1989

EXPECTED COMPLETION DATE:

The field demonstration will end approximately August 26, 1989. The final project report should be completed within six months after the field data collection is completed.

TARGET ANALYTE:

Pentachlorophenol (PCP) in ground water, in treated bioreactor influent, and in bioreactor effluent samples.

DESCRIPTION OF IMMUNOASSAYS:

1. The Rapid Field PCP Immunoassay - This test is a direct competitive enzyme-linked immunosorbent assay (ELISA) that consists of polystyrene microwell strips coated with anti-pentachlorophenol primary antibody. The test involves direct competition for antibody binding between a PCP-enzyme conjugate and PCP in the sample. The test has a detection level of about 3 ppb and a linear dynamic range of about 3-40 ppb. The test is rapid (30 minutes) and easy to perform, and is designed to provide semi-quantitative information about PCP levels. Thus, the method is particularly suitable for field screening.

2. The standard laboratory competitive inhibition enzyme immunoassay (CIEIA) - This is a microtiter plate competitive inhibition ELISA which employs an antigen-coated (dichlorophenol-protein) solid phase. The competition is between analyte (free antigen) in the sample and solid-phase antigen for binding to primary antibody in solution. A secondary antibody conjugated to an enzyme is used as a label to quantitate the amount of primary antibody bound to the antigen on the solid-phase. The method has a detection limit of about 30 ppb and has a linear dynamic range from 30 ppb to 400 ppb. The method is designed to be used for quantitative laboratory analysis of water samples containing PCP.

STANDARD ANALYTICAL METHOD:

The comparison method is gas chromatography/mass spectrometry (GC/MS) by EPA Method 8270 following the EPA Method 3510 extraction protocol. BioTrol, Inc., will also be analyzing selected bioreactor samples by high-pressure liquid chromatography (HPLC). These data will be available for comparison with the immunoassay methods.

KEY REAGENTS:

Rabbit polyclonal antiserum and a pentachlorophenol-enzyme conjugate are used for the rapid field PCP immunoassay. A rat monoclonal antibody and a coating antigen of dichlorophenol-thyroglobulin are used in the standard laboratory PCP immunoassay.

PROJECT DESCRIPTION:

The MacGillis & Gibbs Superfund SITE in New Brighton, Minnesota, is the location of a lumber and pole company where ground water has become contaminated with PCP and polyaromatic hydrocarbons (PAHs). In July 1989, BioTrol, Inc., plans to demonstrate a biological reactor consisting of bacteria that can degrade PCP and PAHs to carbon dioxide, water, and inorganic chloride. In conjunction with the bioreactor demonstration, ground water and effluent samples from within the bioreactor process cycle will be analyzed for PCP and PAHs (and other compounds) using GC/MS, EPA Method 8270.

Westinghouse Bio-Analytic Systems (WBAS) has developed a field portable immunoassay kit for PCP determination. The kit is designed to provide a rapid, sensitive, and inexpensive field screening method. The immunoassay and the bioreactor SITE demonstrations will run concurrently.

The objective of the immunoassay SITE demonstration is to evaluate the ruggedness and utility of the WBAS rapid field immunoassay kit. This will be accomplished by taking grab samples and splits of composite field samples taken for GC/MS analysis as part of the BioTrol reactor demonstration. These samples, along with splits of field blanks, field duplicates, and QA samples, will be analyzed by the field immunoassay kit at the site and by both the field immunoassay kit and the standard laboratory plate ELISA (also developed by WBAS) at two different laboratories, EMSL-LV and WBAS. All field samples selected for on-site analysis (see Appendix I) will be analyzed by the laboratory-based immunoassay, at both laboratories. During the first and third weeks of the immunoassay demonstration, selected field samples will be analyzed by the field immunoassay kit in both laboratories (as well as on site). These analyses will provide laboratory-versus-field site and laboratory-versus-laboratory comparison data. The performance of the rapid field PCP assay will be evaluated and compared with the standard laboratory plate immunoassay and with GC/MS results.

PROJECT ORGANIZATION:

The following diagram summarizes the project organization. Splits of field samples to be analyzed for PCP will be analyzed by the field assay at the site and by both the field assay and the standard laboratory plate ELISA at two laboratories: EMSL-LV and WBAS. Results from each of the methods will be compared. Immunoassay and GC/MS results from different laboratories will also be compared.

Contact persons for participating agencies, contractors, and companies involved are listed below:

Dr. Jeanette Van Emon — EMSL-LV Project Manager
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U.S. Environmental Protection Agency
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Las Vegas, NV 89193-3478
(702) 798-2154

Mr. Richard White or — LESC Technical Lead
Mr. Mark Silverstein — LESC SITE Program Lead
Lockheed Engineering and Sciences Company
Environmental Programs Office, Suite 120
1050 East Flamingo Road
Las Vegas, NV 89119
(702) 798-2165 or
(702) 734-3291

Dr. Steve Soileau Westinghouse Bio-Analytic Systems 15225 Shady Grove Road, Suite 306 Rockville, MD 20850 (301) 921-0031	WBAS Technical Lead
Dr. Herb Skovronek Science Applications International Corporation One Sears Drive Paramus, NJ 07652-3518 (201) 599-0100	SAIC Deputy Project Manager
Mr. Thomas Chresand BioTrol, Inc. 11 Peavy Road Chaska, MN 55318 (612) 448-2515.	BioTrol, Inc., Technical Lead
Ms. Mary K. Stinson Chemical Engineer Releases Control Branch Risk Reduction Engineering Laboratory U.S. Environmental Protection Agency Woodbridge Avenue (MS-104) Edison, NJ 08837-3679 (201) 321-6683	RREL Project Manager
Mr. Gene Easterly U.S. Environmental Protection Agency Environmental Monitoring Systems Laboratory Post Office Box 93478 Las Vegas, NV 89193-3478 (702) 798-2108	EMSL-LV QA Officer
Ms. Susan M. Brustman Public Information Officer Minnesota Pollution Control Agency 520 Lafayette Road St. Paul, MN 55155 (612) 296-7769	State Public Information Officer

The most important responsibilities of participants in the site demonstration are listed below:

1. WBAS will provide sufficient quantities of both the field- and the laboratory-based immunoassay kit reagents to complete the study. In addition, WBAS will provide detailed protocols for both the rapid field and the standard laboratory immunoassays. QA/QC guidelines and performance data are to accompany both immunoassay

method protocols. WBAS is responsible for performing preliminary matrix and analyte stability studies with field samples. WBAS will provide adequate training for Science Application International Corporation (SAIC) contractors in preparing and diluting samples and running the field assay at the demonstration site. WBAS will analyze field sample splits in its laboratory by both the field kit and laboratory-based immunoassay methods and send results to EMSL-LV for timely comparison. WBAS will act as a source of technical support to those performing the immunoassays at the demonstration site and at EMSL-LV.

2. EMSL-LV is responsible for preparing a detailed site demonstration plan and quality assurance project plan (QAPjP). This involves planning, scheduling and coordinating activities associated with the SITE demonstration, and writing a detailed sampling plan. In addition, EMSL-LV will prepare the data quality objectives and design a system for data handling and statistical analysis. EMSL-LV is responsible for analyzing splits of field samples by both immunoassay methods. EMSL-LV will provide confirmatory technical support for developmental work and assay performance evaluation conducted at WBAS. EMSL-LV will write a detailed evaluation report on the technology, which contains thorough statistical comparisons of method data and addresses important QA/QC parameters. EMSL-LV will run a few QA samples (6) and field samples (6) by GC/MS to provide interlaboratory comparison data.

3. SAIC is responsible for performing the field immunoassay analyses. This will involve making splits of composite samples, preparing and diluting samples, analyzing samples, and calculating and recording results. SAIC will label, prepare, and ship split samples, and field assay results, to the two immunoassay laboratories, EMSL-LV and WBAS. SAIC's responsibilities associated with the BioTrol bioreactor demonstration are detailed in the bioreactor SITE demonstration plan (SAIC, 1989). As part of the bioreactor demonstration, SAIC will run GC/MS analysis of each influent and effluent composite sample collected. SAIC will provide GC/ MS results for comparison with the immunoassay results. SAIC will also provide other types of logistical support on-site.

QUALITY ASSURANCE DESIGN AND RESPONSIBILITIES:

The LESC Technical Lead has the responsibility for drafting the QA Plan for the project. Field immunoassay results will be reviewed (checked for errors, completeness, and consistency) by laboratory personnel at both WBAS and EMSL-LV. If problems arise, laboratory personnel will do the necessary experimentation and take appropriate corrective actions. Field personnel will be notified of any necessary corrective actions.

A minimum number of splits from influent and effluent samples as well as two QA audit samples will be interpreted during the preliminary evaluation phase. The data will be reviewed at both immunoassay laboratories. This will provide an opportunity to make necessary corrective actions prior to starting formal data collection.

In the initial stages of the formal data collection (starting on approximately July 24), an auditor (EPA) will perform an on-site technical systems audit. The purpose of these audit is to check that important aspects of the analysis system are operational. These include sample handling; sample tracking and storage; and procedures for diluting, analyzing, and shipping samples.

At EMSL-LV, the field and laboratory immunoassay results will be reviewed and compared to GC/MS results for the six QA audit samples and ten selected field samples. A determination will be made as to whether the immunoassay results satisfy the QC guidelines established for each method.

After all the data have been collected, tabulated, and reviewed and the statistical analysis and comparisons have been done, the final project evaluation report will be prepared. It will contain a summary of all QA/QC activities associated with the project.

Required Quantities: The estimated minimum quantities of reagents necessary for the SITE demonstration are as follows:

1. Approximately 50 field kits, or enough for 15-17 kits for the field analysis, and 15-17 kits for both EMSL-LV and WBAS. Each of these kits should contain enough reagents to run ten 8-well strips.

2. Enough standard laboratory immunoassay reagents to run 30-40 plates, or 15-20 plates for each of the two laboratories, EMSL-LV and WBAS.

The developer, WBAS, has provided assurance that the required quantities of immunoassay supplies and regents have been made and will be available in time for the beginning of the demonstration.

Storage and Stability: Both field kit and standard laboratory kit components are stable for at least several weeks if stored at 4 degrees C. Standards and QA samples will be stored at 4 degrees C in methanol, in sealed ampoules or auto sampler vials with teflon-lined caps. The PCP analyte is very stable under these conditions.

Sample Handling and Cleanup: For the rapid field test, effluent samples will be centrifuged and adjusted in pH, if necessary. In case samples have high concentrations of metal ions, it will be necessary to add EDTA to the buffer used for sample dilution.

For the standard laboratory CIEIA, the standard operating procedure (SOP) supplied by WBAS will be followed. This involves pH adjustment, sample preparation in 25 percent 2-propanol and serial dilutions. Samples with internal spike values outside the acceptable range may undergo cleanup and concentration by solid phase extraction. The effluent samples may require special handling due to biomass and microorganisms which could degrade residual PCP and interfere with immunoassay components. It may be necessary to centrifuge the samples in a table top centrifuge.

As described in the bioreactor SITE demonstration plan, chain of custody procedures will be used for tracking of samples for the bioreactor demonstration. For immunoassay analysis in the two

laboratories (EMSL-LV and WBAS), formal chain of custody procedures will not be necessary. Samples will be carefully inventoried and archived when they are received.

Calibration: A Dynatech microwell strip reader (model number 4025-051) will be used at WBAS and EMSL-LV laboratories and the field site. During the preliminary evaluation phase, these readers will be cross-calibrated with each other and with laboratory-based HP/Genenchem(TM) plate readers. This will be accomplished by making several dilutions of a yellow dye or 2,4-dinitrophenol-glycine at EMSL-LV and sending splits of the dilutions to the other locations for measurement of absorbance at 405 nm on both the strip readers and the laboratory microtiter plate readers. Prior to use, all micropipettors to be used in the study will be checked for accuracy and precision by a gravimetric testing procedure using an analytical balance.

DATA QUALITY OBJECTIVES:

Central Decision or Question: The goal of this study is to evaluate the ruggedness and utility of the WBAS Rapid Field Immunoassay for PCP. The primary objective is to determine whether the rapid field test can provide useful qualitative or semi-quantitative screening information for detecting pentachlorophenol in ground water and for monitoring the effectiveness of remedial action at a Superfund site.

DATA QUALITY OBJECTIVES-ACCEPTANCE CRITERIA:

To be useful as a field screening tool, a field method must be capable of measuring the target analyte with sufficient accuracy and precision to provide criteria for selecting which samples are clearly above, below, or near a critical concentration range. The methods are intended to be used as a supplement to conventional analytical methods used to measure pollutants in environmental samples. Used as a screening tool, these methods can be used in selecting which field samples need to be analyzed by conventional analytical methods. The end result will be a more efficient and cost-effective system for analyzing field samples.

In this study we are limited as to the number and type of samples which can be analyzed. The sampling and analysis scheme had to be designed to integrate into the plan that had been written for the BioTrol Inc. bioreactor SITE demonstration plan. Within the constraints of the study, the goal is to evaluate the ruggedness and utility of the field immunoassay method as a rapid field screening tool. The other key objective is to obtain information regarding the limitations and range of applicability of the method. The sampling design chosen for the immunoassays is sufficient to achieve these goals.

To adequately demonstrate the utility of the field PCP immunoassay as a field screening method, the following data quality objectives are proposed:

1. For field samples, the field immunoassay test result should not differ from the GC/MS result by more than a factor of two.

2. The maximum coefficient of variation (CV) for the QC performance standards (20 ppm), which are diluted and run by different operators on different days, should not exceed 50 percent.

3. The QA audit sample field test result should be within ±50 percent of the expected value.

These criteria are proposed as guidelines for evaluating the quality and validity of the semi-quantitative (or qualitative) information obtained from the test results. If the data do not satisfy the objectives stated here, the basic objectives of the SITE demonstration may still be met. Useful information regarding the limitations and applicability of this particular method for field testing will have been obtained. The outcome and conclusions will be discussed in the final evaluation report.

SEVEN ELEMENTS OF DATA QUALITY:

Critical Concentration Range: The critical concentration range of PCP analyte for this study is approximately 0.2 ppm to 50 ppm. The ground-water samples flowing into the bioreactor will contain approximately 45-50 ppm PCP. Effluent samples will vary in concentration. If the reactor is working as expected, final effluent PCP values should range from 0 to about 1 ppm. Effluent PCP levels may vary with flow rate through the reactor.

The Metropolitan Waste Control Commission (MWCC) standard value for release of PCP is 1 ppm. Bioreactor effluent, after solids removal and carbon filtration, must fall below 1 ppm before release. The Minnesota Pollution Control Agency (MPCA) standard for PCP in water is 0.22 ppm.

Required Sensitivity: Both the rapid field and the standard laboratory immunoassays are sensitive into the parts per billion (ppb) range. Both methods are sensitive to below the MWCC and MPCA standards.

Environmental Samples: The ground-water samples taken from one well at the MacGillis and Gibbs site contain PCP (40-50 ppm) and polyaromatic hydrocarbons. The samples may contain iron and other metal ions. The measured pH is about 6.8. The influent may have to be run through an oil/water separator prior to treatment. The influent samples will be adjusted to pH 7.4 and 25 degrees C, and nutrients will be added prior to flowing into the reactor. Splits of composite influents for GC/MS and immunoassay will be taken at this point.

Site Selection: The MacGillis and Gibbs site is an ideal location for a demonstration of the WBAS Rapid Field Immunoassay Kit because the ground-water samples are relatively clean (e.g., low in sediment or turbidity) and contain high (ppm) concentrations of PCP. Since the sampling and analysis plan was already in place for the BioTrol bioreactor demonstration, it was not necessary or practical to design a sampling and analysis scheme from scratch. The bioreactor demonstration provides an opportunity to demonstrate the ability of the immunoassay method at a site where remedial actions are under way, and where comparative Contract Laboratory Program (CLP) data is being collected.

Field Sampling Design: The sampling design for the immunoassay demonstration will coincide with that set forth in the BioTrol bioreactor demonstration. Selected samples which are taken for GC/MS analysis will be split and run by both the rapid field and the standard laboratory assays. The first part of the bioreactor SITE demonstration will consist of three two-week phases, and for each, the reactor will be run at a different flow rate. Composite samples will be collected for GC/MS analysis six days a week. Immunoassay analysis of key samples is listed in Appendix I. The number of splits, QA standards, duplicates and blanks selected are sufficient to provide statistically valid conclusions from the data (Appendix I).

The following provides a brief description of each sample type and the total number that will be analyzed. Approximately 18 influent and 12 effluent samples will be run during the three weeks immunoassay samples are being run. An additional 1 or 2 influent and effluent samples will be run during the preliminary evaluation phase.

Environmental Samples: Splits of selected grab samples and composite influent and effluent samples taken for GC/MS analysis will be assayed using the rapid field immunoassay and the standard laboratory CIEIA in the laboratories (EMSL-LV and WBAS). During the first and third weeks of the immunoassay demonstration, selected field samples will be analyzed using the field immunoassay kit in both laboratories. Selected samples (see Appendix I) will be run by the rapid field immunoassay, on site. Each sample will be run at several dilutions, both for range finding and in duplicate at optimal dilution, with and without an internal standard spike. Each day, field blanks (instrument water rinses) will be run undiluted and diluted ten fold. In addition, on a daily basis, a minimum of 2 to 3 negative controls (in buffer from the kit) will be run undiluted and a single dilution of a 20 ppm performance standard will be run on one strip. This 20 ppm standard will be prepared at EMSL-LV and will be sent to the field and to the WBAS laboratory. It will be run as a known standard, diluted to an optimal level, and run in single wells on one strip, on separate days.

On two days each week, field duplicate pairs (influent on one day, effluent on the other) will be run in duplicate in both the rapid field and standard plate ELISA. If possible, these field duplicates will be the same ones selected by SAIC for GC/MS analysis in order to provide a direct comparison.

On one day each week, a grab sample of raw (untreated) ground water will be taken. SAIC will analyze a split of this sample by GC/MS. Splits of this sample will be analyzed by the rapid field immunoassay on site, and by both immunoassay methods in the two laboratories.

QA Samples: The same QA samples (single analyte PCP and mixed phenols, with PCP) will be analyzed by all three methods: the field immunoassay, the laboratory immunoassay and GC/MS. These will be prepared from single lots of EMSL-Cincinnati performance evaluation samples (of each type) by dilution in methanol. These dilute QA samples will be prepared at EMSL-LV, placed in ampules and sent to the field site, WBAS, and to SAIC (for GC/MS analysis).

On three days each week, several dilutions of the PCP QA samples (two single analyte and one mixed phenols) will be analyzed using the field assay on-site, using both the laboratory and the field immunoassays, at WBAS and EMSL-LV. These samples will be run semi-blind using the field

immunoassay on-site. That is, the recommended dilutions (but not the concentrations) will be provided.

At least six ampules of the same diluted QA samples prepared for immunoassay analysis will be analyzed by GC/MS at both SAIC and EMSL, during the study. At least six splits of field samples will be analyzed by GC/MS at the EMSL-LV laboratory.

Controls and Blanks: Each day on the first strip run, an instrument rinse field blank will be run at two different dilutions and a negative control (1X buffer from kit) will be run in duplicate. The field blank will consist of a laboratory water rinse of the ISCO composite sample collector, after it has been cleaned (SAIC, 1989).

Internal Standard Spikes: Each effluent field sample split will be diluted and run in duplicate with and without a 15 ppb internal standard. This will be done after determining the proper sample dilution by running 2-3 range-finding dilution strips. One daily influent sample per week will also be run in duplicate, with and without a 15 ppb internal standard spike.

QC Performance Standards: Each day, one 20 ppm performance standard will be run at a single dilution on one strip in the field immunoassay, on-site. This will provide a minimum of 18 replicates of a known standard for charting and precision analysis.

KEY INTERFERENCES:

Ground-water samples may contain iron and other metal ions, polyaromatic hydrocarbons, mixtures of phenols, and possibly oil. The extent of interference from these sources is estimated to be minimal, since large dilutions of samples will be done prior to analysis. The effluent samples will contain biomass and bacteria, inorganic and organic nutrients, and a mixture of unknown metabolites. The effects of other compounds in the matrix on the immunoassay is unknown and will have to be studied as part of the preliminary evaluation phase.

SPECIFICITY:

The rabbit polyclonal antibody used for the field PCP immunoassay method cross-reacts with 2,3,5,6-tetrachlorophenol (19 percent), several trichlorophenols (7 percent), and tetryhydroquinone (11 percent). Phenol and dichlorophenols do not interfere significantly (see Table A-1). The rat monoclonal antibody used for the laboratory PCP immunoassay cross-reacts with 2,3,5,6-tetrachlorophenol (42 percent), 2,4,6-trichlorophenol (12 percent), and 2,3,5,6-trichlorophenol (8.8 percent). As with the rabbit polyclonal, the monoclonal does not cross-react significantly with phenol or dichlorophenols (see Table A-2).

ASSESSMENT OF DATA QUALITY:

Statement of Intended Use: The data obtained in this SITE demonstration is to be used by the EPA to evaluate the ruggedness and utility of the WBAS field PCP immunoassay. The objective is

TABLE A-1. PENTACHLOROPHENOL FIELD ANALYSIS KIT CROSS-REACTIVITY PROFILE

CHEMICAL	PERCENT CROSS-REACTIVITY
PENTACHLOROPHENOL	–
2,3,5,6-TETRACHLOROPHENOL	19
2,4,6-TRICHLOROPHENOL	7
2,3,6-TRICHLOROPHENOL	7
2,4,5-TRICHLOROPHENOL	0.4
2,6-DICHLOROPHENOL	0.7
TETRYHYDROQUINONE	11
2,3,4-TRICHLOROPHENOL	2.5
2,4-DICHLOROPHENOL	<0.1
2,5-DICHLOROPHENOL	<0.1
3,5-DICHLOROPHENOL	<0.1
3,4-DICHLOROPHENOL	<0.1
2,3-DICHLOROPHENOL	0.2
4-CHLOROPHENOL	<0.1
PHENOL	<0.1
PENTACHLOROBENZENE	<0.1
2,3-DINITROTOLUENE	<0.1
2,4-DINITROTOLUENE	<0.1
2,4,5-TRICHLORONITROBENZENE	<0.1

to determine whether this immunoassay can provide qualitative or semi-quantitative field screening information, which is useful for site characterization and monitoring remedial actions at Superfund sites. The end result of this and possible future SITE demonstrations for this technology will be a comprehensive report which covers specific recommendations and limitations for use.

Accuracy: Accuracy will be assessed by comparison of the field assay results with those expected for QA audit sample spikes and performance standards. Accuracy will also be evaluated by comparison of the field immunoassay results with those obtained by GC/MS and the

TABLE A-2. SPECIFICITY OF PCP MONOCLONAL ANTIBODIES

Compound	Molar IC_{50}[a]	Cross-Reactivity with PCP[b]
Pentachlorophenol	$2.2 (\pm 0.3) \times 10^{-6}$	—
2,3,5,6-Tetrachlorophenol	$5.3 (\pm 0.6) \times 10^{-6}$	42.0
2,4,6-Trichlorophenol	$1.8 (\pm 0.3) \times 10^{-5}$	12.0
2,3,6-Trichlorophenol	$2.5 (\pm 0.1) \times 10^{-5}$	8.8
2,6-Dichlorophenol	$1.2 (\pm 0.1) \times 10^{-4}$	1.8
Tetrachlorohydroquinone	$2.8 (\pm 0.1) \times 10^{-4}$	0.8
2,3,4-Trichlorophenol	$4.5 (\pm 0.3) \times 10^{-4}$	0.5
2,3,5-Trichlorophenol	$4.3 (\pm 0.3) \times 10^{-4}$	0.5
2,4-Dichlorophenol	NI[c]	0
2,5-Dichlorophenol	NI	0
3,5-Dichlorophenol	NI	0
3,4-Dichlorophenol	NI	0
2,3-Dichlorophenol	NI	0
4-Chlorophenol	NI	0
Phenol	NI	0
Pentachloroaniline	NI	0
Pentachlorobenzene	NI	0
2,3-Dinitrotoluene	NI	0
2,3-Dinitrotoluene	NI	0
2,4-Dinitrotoluene	NI	0
2,4,5-Trichloronitrobenzene	NI	0

[a] Molar concentration of compound that inhibits 50 percent antibody binding in the "normal" competitive inhibition enzyme immunoassays.
[b] [IC_{50} PCP/IC_{50} compound] x 100
[c] NI = Not inhibitory; 1.0×10^{-3} M

Source: Courtesy of WBAS

laboratory immunoassay method. Confidence intervals for each methods test result will be determined and the degree of overlap will be quantitated.

Precision: The precision of the field immunoassay method has been evaluated by having two analysts run six replicates of each kit standard (five different levels). Field kit precision on-site will be evaluated by repetitive analysis of blanks, 20 ppm performance standards (diluted), and QA audit samples. A minimum of six single analyte PCP QA samples and three phenols mixture (with PCP) QA audit samples will be analyzed for the entire study. A minimum of 18 replicates of the performance standard (single dilution) and the negative control (one X sample dilution buffer) will be run.

Each week one field duplicate pair of one influent and one effluent sample will be analyzed on duplicate strips. In addition, duplicates of each effluent tested will be run, diluted to 3-20 ppb, and with and without internal standard spikes (15 ppb).

Internal Standard Spike Recovery: The internal standard spike recovery data for the field immunoassay is intended to provide information on the relative levels of matrix interference in the field samples after dilution to the appropriate concentration range.

For the standard laboratory CIEIA, the internal standard spike recovery will be used as a QC check on the matrix effects. Spiked samples that do not fall within ±25 percent of the expected value may be cleaned up by solid phase extraction and reanalyzed.

Detection Limit: The detection limit for the field immunoassay test is defined as follows: any sample with an absorbance greater than 90 percent of the absorbance range should be reported as less than the Method Detection Limit (MDL). The approximate MDL is 1-3 ppb of PCP. The MDL for the standard laboratory CIEIA is defined the same way: any sample with an absorbance greater than 90 percent of the absorbance range should be reported as less than the MDL. The approximate MDL is 30 ppb of PCP.

(NOTE: MDLs provided by manufacturer; no official MDL studies have been performed).

Representativeness: See the document entitled "Quality Assurance Plan for Immunoassay Evaluation and Research Projects" (White and Miah, 1989) for a general discussion. For this SITE demonstration, the field samples consist of PCP-contaminated groundwater samples and bioreactor effluent samples. The groundwater samples may or may not be representative of groundwater taken at other wells or sites. The effluent samples from the bioreactor are representative of the type of matrix that would be commonly found with this type of remedial action. The immunoassay demonstration at this site is limited to the sampling design chosen for the bioreactor demonstration. Although the data collected will be representative of the utility of the test at a single site, it may be necessary to sample water from a variety of wells (and sites) in future demonstrations to show the full capability of the method.

Completeness: See the QA document mentioned above (White and Miah, 1989) for a discussion of this element of data quality as it refers to immunoassay projects. The completeness objective of 90 percent (of the expected number of samples) established for immunoassay projects in general is also applicable for this SITE demonstration.

Comparability: See the QA document mentioned above (White and Miah, 1989) for a discussion of this element of data quality as it refers to immunoassay projects. To meet EPA requirements, study data must be reported in the same units as other projects using the same technology. For this SITE demonstration, comparability will be addressed by comparing the on-site field immunoassay results with the standard laboratory immunoassay results obtained at two laboratories and the GC/MS results run at one or two laboratories. WBAS has written a detailed SOP containing QC protocols and acceptance criteria for the standard laboratory immunoassay and the solid phase extraction procedure. WBAS has sent a protocol for the field PCP kit which contains a QC protocol and strip acceptance criteria (see Appendix II). Copies will be sent to all parties involved and strict adherence to the immunoassay test and QC protocols will be stressed.

The field immunoassay kit protocol is easy to learn and WBAS will train the SAIC contractors who will be performing the test in the field. WBAS will send kits and a detailed protocol to SAIC. After SAIC performs the immunoassay, WBAS will discuss questions and problems with them. An SAIC representative will travel to WBAS for more in depth training during the weeks prior to the start of the demonstration. A representative from WBAS will be on-site to provide assistance during the first 2-3 days of the formal demonstration.

Specificity/Interference: A phenols mixture (containing PCP) QA audit sample will be analyzed using the field immunoassay on-site and at both laboratories by the standard laboratory immunoassay. At least three of these will be analyzed during the demonstration period. If possible, depending on laboratory commitments, these standards will also be analyzed by GC/MS by both SAIC and EMSL-LV. Analysis of this mixture will provide information on the specificity of the antibodies used for the immunoassay methods (also see Tables A-1 and A-2).

All field samples analyzed by the laboratory immunoassay will be run with and without internal spikes. A minimum number (3) of field influent and all effluent samples will be analyzed with and without internal spikes. This will provide information regarding the extent of sample matrix interference. Possible matrix effects and interferences will be studied at WBAS and EMSL-LV on representative groundwater, effluent, and surface water samples shipped from BioTrol, Inc. during the preliminary evaluation phase of the bioreactor. The most effective sample pretreatment steps for influent and effluent field samples will be determined by WBAS during the preliminary evaluation phase. The immunoassay field kit protocol and training sessions will address all necessary sample pretreatment steps.

CORRECTIVE ACTIONS:

In the event that data from the field immunoassay analysis of QA samples, performance standards, or blanks fall outside the expected ranges (contained in data quality objectives and the QC guidelines of the kit protocol) the strip analysis will be repeated. If the problem persists, splits of field samples will continue to be made for immunoassay analysis, but field testing may be suspended until WBAS and EMSL-LV laboratories can do sufficient testing to determine the source of the problem. A minimum of 20-30 mL of each field sample will be archived at both laboratories and on-site. Samples will be stored (at 4 degrees C) until the study is completed in case it is necessary to reanalyze selected samples.

PERFORMANCE AND SYSTEMS AUDITS:

Performance Audit: Ongoing assessment of data quality will be accomplished through analysis of a minimum of nine semi-blind (with only the recommended dilutions given) QA samples (reference standard materials obtained from EMSL-Cincinnati). At least three of these will be mixed phenols standards (EMSL-Cin reference standard C-090-02, acid extractables II, in methylene chloride). Ampoules of the QA samples will be made by EMSL-LV and distributed to the field site and to WBAS. The same nine QA samples (6 PCP only [EMSL-Cin reference standard EV-062-03-13 in methanol] and 3 mixed phenols with PCP) will be assayed in the field PCP immunoassay at the site and both the field and laboratory immunoassays at WBAS and

EMSL-LV. An external reviewer has the responsibility for comparing the results from both the field and laboratory methods with the expected results for the QA audit samples.

Site Systems Audit: At some time during the initial stages of the formal data collection, an on-site systems performance audit will be conducted by an individual who is familiar with the technology and who has been provided with a check list of critical procedural items. This list will be prepared by EMSL-LV. The results of the audit will be summarized in the final project evaluation report.

PRELIMINARY EVALUATION PHASE:

A minimum of two QA samples (one mixed phenols, one PCP only) and 2-3 influent and effluent samples from the field (if available) will be analyzed by both immunoassay methods. Results from the two methods will be compared with the expected results for standards or with HPLC results for field samples. This will provide an opportunity to take any necessary corrective actions, before the formal data collection starts.

QUALITY CONTROL PROTOCOLS:

QC protocols for the standard laboratory PCP immunoassay are detailed in the SOP for the method and in the "Quality Assurance Plan for Immunoassay Evaluation and Research Projects" (White and Miah, 1989).

WBAS has provided QC protocols for the field immunoassay (see Appendix II). They have done a solid-phase variability check on the strips and will cross-calibrate the field- and laboratory-based strip readers, as will EMSL-LV.

QC protocols for the solid phase extraction method are contained in the SOP for the laboratory PCP immunoassay method.

The QC protocols contained in EPA method 8270 will be used for GC/MS analysis at both SAIC and EMSL-LV.

DATA HANDLING:

A field form will be used for identification and tracking of samples. A draft copy is included in Appendix II. This form contains all the necessary information regarding the details of collection, storage, time, date, and operator; as well as the analytical results. A copy of this form will be sent to the WBAS and EMSL-LV laboratories. The reporting units for the immunoassay data are parts-per-million (ppm) or ug/ml. Field and laboratory analysis data will be stored in personal computers (PC), and backup copies will be made on floppy disks.

For the field immunoassay, standard curve data points will be plotted on semi-log graphs and fitted by hand. The same data can also be manually entered into a PC in the laboratory and fitted by other methods, for comparison.

Laboratory immunoassay results will be handled with the Hewlett-Packard Titer-Calc® software with 4-parameter curve fitting. Data will be stored in the computer and on backup disks.

DATA VALIDATION, REDUCTION, AND REPORTING:

On-site, field PCP kit data will be calculated from semi-log plots of kit standards made for each eight well strip. The field forms will then be sent daily to both WBAS and EMSL-LV laboratories, where the field data will be initially reviewed, as received, for completeness, consistency, and falling within expected ranges. The data are keyed into a PC using a standard spreadsheet or statistical software. Backup copies will be kept on floppy disks. Any necessary corrective actions (e.g., confirmation of suspect data) will be taken, and then selected samples will be reanalyzed by the field kit in the laboratory. Samples are prepared as necessary and analyzed using the laboratory PCP immunoassay. If samples with the internal standard spikes are outside of specification, samples will be reanalyzed after cleanup. Data from both the laboratory and field PCP immunoassay analyses will be entered into the computer data base. All data entries will be checked for accuracy by a second party. Data received from the GC/MS analyses at both SAIC and EMSL-LV will be reviewed and entered into the computer data base for comparison with the immunoassay data. After further detailed review by the project leader and EPA Technical Monitor for technical validity, such as incorrect number of significant figures or inconsistency in results for the same sample analyzed at different locations or at difference dilutions, selected samples will be reanalyzed after necessary corrective actions are taken.

The data from GC/MS analysis of the 6-9 QA audit samples and the six selected field samples will be reviewed. If necessary, samples will be reanalyzed by GC/MS.

All immunoassay and GC/MS data entered into the computer data base will be organized and tabulated for easy comparison. The PC will be interfaced with the VAX mainframe computer and the data will be analyzed using Statistical Analysis System (SAS) and other available statistical computer software.

A final report containing tabulated data, interpretations, conclusions, and QA/QC summary will be prepared. The report will be reviewed by the EPA Technical Monitor and circulated for internal peer review. The data may also be published or presented at scientific meetings for external peer review.

STATISTICAL EVALUATION:

The data analysis will consist of two phases. In the first phase the quality of data will be assessed. This will consist of a number of standard procedures. QA/QC data will be examined (via time trends and descriptive statistics) to ensure that consistent results and adequately accurate measurements are available. This will require information from the various blanks, audit

samples, splits and duplicates. This form of analysis will be carried out for each analysis method at each site. Also, based on analysis splits and within- and between-strip replicates, a simple analysis of variance (ANOVA) may be performed to isolate major components of error.

The second phase of analysis will be the comparison between methods/sites. Of primary concern will be the comparison between routine GC/MS values and the field strip test results. Given positive results from the first phase of analysis, we will assume that the GC/MS results are the "true" values with respect to the accuracy expected from the immunoassay method. The comparison will be looked at via summary statistics, t-tests for differences, correlation of results, and XY plots. Similar analyses are planned for comparing the results from the laboratory-based immunoassay method with the GC/MS results, although the primary focus will be on the field method. We will also try to estimate whether laboratory bias is significant and whether method to method bias exists.

In summary, the data analysis methodology is not overly complicated. However, the number of inter-laboratory and inter-method comparisons involved will amplify the amount of time required to complete the analysis. See Appendix III for equations used to calculate statistics.

REPORTS FOR MANAGEMENT:

A short summary report will be written at the conclusion of the preliminary evaluation phase. The report will contain a comparison of the data obtained by the rapid field method and the laboratory method in the two laboratories. Results obtained by both methods on the two QA audit samples (one PCP only, one mixed phenols, containing PCP) will be compared with the expected results for the standards and the GC/MS results from at least one laboratory. Laboratory and field immunoassay results will also be compared with BioTrol HPLC results, whenever possible. The report will contain a brief assessment of the quality of the data, as well as any significant problems encountered. Approaches to alleviate or eliminate problems will be discussed.

The final project evaluation report will follow the guidelines written for project final reports within the "Quality Assurance Plan for Immunoassay Evaluation and Research Projects" (White and Miah, 1989) as it applies to the SITE evaluation report. This report also follows the current guidelines for SITE evaluation reports. The report will contain detailed analysis and interpretation of the data collected and a summary of the quality assurance activities associated with the project. A copy of the final report will be reviewed by the EPA Technical Monitor and other EPA and LESC managers involved with the project. The report will be subject to both internal and external peer review.

APPENDIX I
SAMPLING AND ANALYSIS SCHEDULE
FOR THE FIELD PCP IMMUNOASSAY KIT SITE DEMONSTRATION

PRELIMINARY EVALUATION (bioreactor start-up) 26 days, June 26-July 21.

PART A-(bioreactor flow rate= 1 gal/min) 6 days, July 24-29

PART B-(bioreactor flow rate= 3 gal/min) 6 days, August 7-12

PART C-(bioreactor flow rate= 5 gal/min) 6 days, August 21-26

Use the same sampling and analysis scheme for all three parts. Effluent samples are from reactor 3 (final), unless otherwise stated. The samples referred to as the routine influent or effluent is a split of the composite sample taken for GC/MS analysis, for that day.

The routine influent will be taken from the holding tank, containing treated (nutrients added, pH adjusted to 7.2) well water. In addition, once a week, a grab sample of raw or untreated well water will be taken for GC/MS analysis. A split of this grab sample is to be analyzed by the field PCP immunoassay method also. If possible, do the analysis schemes in the order listed: DAY 1 first, DAY 2 second, and DAY 3 third. The short day routines are for days when the person doing the field analysis is busy with other activities and the long day routines are for days when there is more time available. The 4 kit standards that are run on each strip are the following: 3 ppb, 7.1 ppb, 16.9 ppb, and 40 ppb. Each day, run the QC STRIP first.

All samples should be run as soon as possible after collection. Try to run all samples within a 24 hr period after splitting. Try to complete the analysis of all the scheduled samples (see the weekly totals list below) within each of the three 6 day periods. A minimum volume of 20 ml from each field sample analyzed should be placed in a 30 ml amber vial, labelled with an SAIC label (see example below) and capped tightly with a teflon lined screw cap. All these vials are to be stored at 4 degrees C, in case later analysis is necessary. If time does not permit the analysis of all the above samples during the 6 day week, unanalyzed samples are to be run on the following week.

	QC STRIP
LONG DAY 1	1 strip with a field water blank, undiluted and diluted 1 to 10, a negative control (1X kit dilution buffer) and a 20 ppm performance standard, diluted 1 to 1000 + 4 kit standards.

EFFLUENT STRIPS

1-2 strips with four semi-log serial dilutions (suggested dilutions: 1 to 10, 1 to 50, 1 to 100, and 1 to 500) of the routine effluent + 4 kit standards. 1 strip with the routine effluent diluted to about 10 ppb (3-20 ppb) run in duplicate with and without a 15 ppb internal standard spike + 4 kit standards.

QA SAMPLE STRIPS

2 strips with three serial two-fold dilutions of a semi-blind (see instructions) type A QA audit sample + 4 kit standards, and one negative control.

INFLUENT STRIP

1 strip with routine influent at 4 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, 1 to 4000 and 1 to 8000) + 4 kit standards.

LONG DAY 2 - SAME AS LONG DAY 1 .

LONG DAY 3 - SAME AS LONG DAY 1, EXCEPT RUN TYPE B QA AUDIT SAMPLE.

QC STRIP

SHORT DAY 1 1 strip with a field water blank, undiluted and 1 to 10 diluted, a negative control (1X kit dilution buffer) and a 20 ppm performance standard, diluted 1 to 1000 + 4 kit standards.

INFLUENT STRIPS

2 strips with routine influent at 3 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, and 1 to 4000) + 4 kit standards, and one negative control.

2 strips, with duplicates of both the routine influent and its field duplicate (split), each diluted to near the midpoint (20+/- 10 ppb) of the standard curve + 4 kit standards.

1 strip with split of the routine influent diluted to about 10 ppb (3-20 ppb) and run in duplicate with and without a 15 ppb internal standard spike + 4 kit standards.

QC STRIP

SHORT DAY 2

1 strip with **field water blank**, undiluted and diluted 1 to 10, 1 **negative control** (1X kit dilution buffer) and a 20 ppm **performance standard**, diluted 1 to 1000.

EFFLUENT STRIPS

2 strips each with a split of the routine effluent at 4 dilutions (suggested dilutions: 1 to 10, 1 to 100, 1 to 50 and 1 to 500) + 4 kit standards.

2 strips, with duplicates of both the routine effluent and its field duplicate split, each diluted to near the midpoint of the standard curve (20+/-10 ppb) + 4 kit standards.

INFLUENT STRIP

1 strip with the routine influent at three dilutions (suggested dilutions: 1 to 1000, 1 to 2000, and 1 to 4000) + 4 kit standards and one **negative control**.

QC STRIP

SHORT DAY 3

1 strip with a **field water blank**, undiluted and diluted 1 to 10, a **negative control** (1X kit dilution buffer) and a 20 ppm **performance standard** diluted 1 to 1000 + 4 kit standards.

INFLUENT STRIPS

2 strips with split of the routine influent at 3 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, 1 to 4000) + 4 kit standards, and one **negative control**.

1 strip with split of the routine influent diluted to about 10 ppb (3-20 ppb) and run in duplicate with and without a 15 ppb internal standard spike + 4 kit standards.

DAY FOR INFLUENT GRAB - After running the other strips scheduled for this day, run the following.

INFLUENT STRIPS

2 strips with split of the raw influent grab sample at 3 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, 1 to 4000) + 4 kit standards, and one negative control.

WEEKLY TOTALS

1) 6 routine (daily) influent samples

2) 4 routine (daily) effluent samples

3) 1 field duplicate split of a routine (daily) effluent

4) 1 field duplicate split of a routine (daily) influent

5) 1 raw (untreated) influent grab sample

6) 2 type A QA audit samples

7) 1 type B QA audit sample

8) 6 routine (daily) field water blanks (equipment rinse)

9) 6 daily replicates of the 20 ppm QC performance standard, diluted fresh each day.

10) A minimum of 18 negative controls (1X dilution buffer from kit)

APPENDIX II

Westinghouse
Bio-Analytic System

PENTACHLOROPHENOL FIELD ANALYSIS KIT

SUMMARY

The Pentachlorophenol Field Analysis Kit is an immunoassay-based kit that is used for the detection of pentachlorophenol (PCP) in water. The detection limit of the assay is approximately 1 parts per billion (ppb). However, the detection limit of the assay for the purposes of the BioTrol field test is 3 ppb. The total time to complete the test is approximately 30 min (not including sample preparation time).

NOTE: Please read all of the instructions completely before beginning your first assay.

CONTENTS

Each PCP Field Analysis Kit should contain the following:

1) Rinse Solution (one bottle)

2) Eight-Well Strips (5)

3) PCP Standards (one bottle each)

   1 part per million (ppm)
   40 ppb
   16.9 ppb
   7.1 ppb
   3 ppb

4) Negative Control (one bottle)

5) 100X Buffer (one bottle)

6) Substrate (one bottle)

7) Chromogen (one bottle)

8) Stop Solution (one bottle) CAUTION: CONTAINS 0.3N SULFURIC ACID!!!

9) Enzyme Conjugate (one bottle)

10) Instructions (one set)

If any reagents are missing, please contact Westinghouse Bio-Analytic Systems Company at (301) 670-0688.

Sample dilution

1) Influent - The initial PCP concentration of the influent sample should fall between 50 ppm and 1 ppm. The following dilutions will permit the sample concentration to be estimated if the sample PCP concentration is within this range.

   a) Label four tubes with the sample number. Label one of the four tubes with '1:10', one with '1:100', one with '1:1000', and one with '1:10000'.

   b) Add 0.9 ml 1X buffer to each of the four tubes.

   c) Using a 200 microliter single channel pipettor, transfer 100 microliters of the 'undiluted' sample to the tube labelled '1:10'. Mix well, using the single channel pipettor.

   d) Using the same single channel pipettor and tip, transfer 100 microliters of the '1:10' sample to the tube labelled '1:100'. Mix well, using the single channel pipettor.

   e) Using the same single channel pipettor and tip, transfer 100 microliters of the '1:100' sample to the tube labelled '1:1000'. Mix well, using the single channel pipettor.

   f) Using the same single channel pipettor and tip, transfer 100 microliters of the '1:1000' sample to the tube labelled '1:10000'. Mix well, using the single channel pipettor.

   g) Analyze the samples labelled '1:100', '1:1000', and '1:10000' with the PCP Field Analysis Kit.

1) Effluent - The initial PCP concentration of the effluent sample should fall between 1 ppm and 100 ppb. The following dilutions will permit the sample concentration to be estimated if the sample PCP concentration is within this range.

   a) Label three tubes with the sample number. Label one of the three tubes with '1:10', one with '1:32', and one with '1:100'.

   b) Add 0.9 ml 1X buffer to the tube labelled '1:10' and 1.0ml 1X buffer to the tubes labelled '1:32' and '1:100'.

   c) Using a 200 microliter single channel pipettor, transfer 100 microliters of the 'undiluted' sample to the tube labelled '1:10'. Mix well, using the single channel pipettor.

   d) Using a 1000 microliter single channel pipettor and tip, transfer 462 microliters of the '1:10' sample to the tube labelled '1:32'. Mix well, using the single channel pipettor.

e) Using a 1000 microliter single channel pipettor and tip, transfer 462 microliters of the '1:32' sample to the tube labelled '1:100'. Mix well, using the single channel pipettor.

f) Analyze the samples labelled '1:10', '1:32', and '1:100' with the PCP Field Analysis Kit.

3) Quality Control Samples - The general procedure for dilution of quality control samples is as follows. If the concentration of the sample is known, prepare serial dilutions of the sample (be sure no single dilution is greater than 1:10) until the sample is diluted to 30 ppb. Label the tube with the sample number and '30 ppb'. Prepare two 1:1 serial dilutions of the 30 ppb sample in 1X buffer and label the tubes '15 ppb' and '7.5 ppb'. If the concentration of the sample is not known, treat the sample as you would an influent sample.

ANALYSIS KIT PROCEDURE

The time required to run test is approximately 30 minutes.

1. Remove strip(s) needed from the resealable bag. NOTE: Strips are not reuseable. Remove the reagents from the box and allow them to equilibrate at ambient temperature.

   Place the strip(s) in the supplied strip holder. The square end of the strip should be placed at the top of the holder (The side with raised numbers). The individual wells will be identified by the letters that appear on the left side of the strip holder.

2. Choose an appropriate strip format for your needs (Supplied in the sampling protocol). Using a 200 microliter single channel pipettor, add 50 microliters from the standard bottles and appropriate sample test tubes to the appropriate wells. Be sure and change pipet tips between each standard and sample.

3. Using a 200 microliter single channel pipettor, add 50 microliters of the Enzyme Conjugate bottle to all the wells being used.

4. Gently tap the strips to mix the reactants; be careful not to splash reagents out of the wells or into adjacent wells.

5. Wait 15 minutes. Tap the strips once or twice during this time. During the incubation step, prepare the substrate solution. Using a 1000 microliter single channel pipettor, add 220 microliters of the Substrate and 220 microliters of the Chromogen to a test tube for each strip that is being used. For example, if one is using 3 strips, add 660 microliters of Substrate and 660 microliters of Chromogen to a test tube. Mix well.

6. Shake out contents of the strip into the sink or other suitable container. Rinse the strips with the squirt bottle provided by filling and emptying each strip four times. Because the Rinse Solution contains a detergent, a large amount of bubbles may form in the wells and make it more difficult to adequately wash the strips. If this occurs, tap the strips onto absorbent paper between washes. This should reduce the number of bubbles.

   Note: The strip(s) should be taped securely into the holder along the top and bottom edges. This will keep the strips from coming loose from the holder during the washing step.

7. Invert the strip and dry by tapping several times on a paper towel.

   Note: Do not wipe the wells with a towel or heat the strip to dry the inner part of the wells. It is not necessary to completely dry the wells, but they should be tapped as dry as possible.

8. Using a 200 microliter single channel pipettor, add 100 microliters of the substrate solution (Prepared in step 5) to each well.

9. Wait 15 minutes and stop the color reaction by adding the Stop Solution. Using a 200 microliter single channel pipettor, add 50 microliters of the Stop Solution to each well. Touch the pipet tip to the side of each well when dispensing and do not allow the tip to go below the liquid level; this could contaminate the stop solution in the bottle when the tip is reintroduced into the bottle. Tap the strips gently to effect mixing and read the strips with the portable strip reader. Be sure the reader contains the 405 nm filter. Please note that the substrate solution should change from a blue color to a yellow color.

   Note: Caution should be used as the Stopping Reagent contains Sulfuric Acid.

10. The approximate PCP concentration of the samples can be determined by comparing the sample colors to the standard colors. The rate of color development is inversely proportional to the concentration of PCP in the sample. Plot the standards on the sample sheets supplied by SAIC. Fit the best straight line to the standards (by eye) and calculate the sample concentrations using the best-fit line. Multiply the concentration obtained by the dilution factor to obtain the undiluted sample concentration.

QUALITY CONTROL

STORAGE

Keep the kit refrigerated when not in use.

TO OUR CUSTOMERS:

Westinghouse Bio-Analytic Systems Co. wants to hear from you. Should you have any difficulties, questions, or comments about this product, please contact Dr. Stephen D. Soileau at (301) 670-0688 or write to:

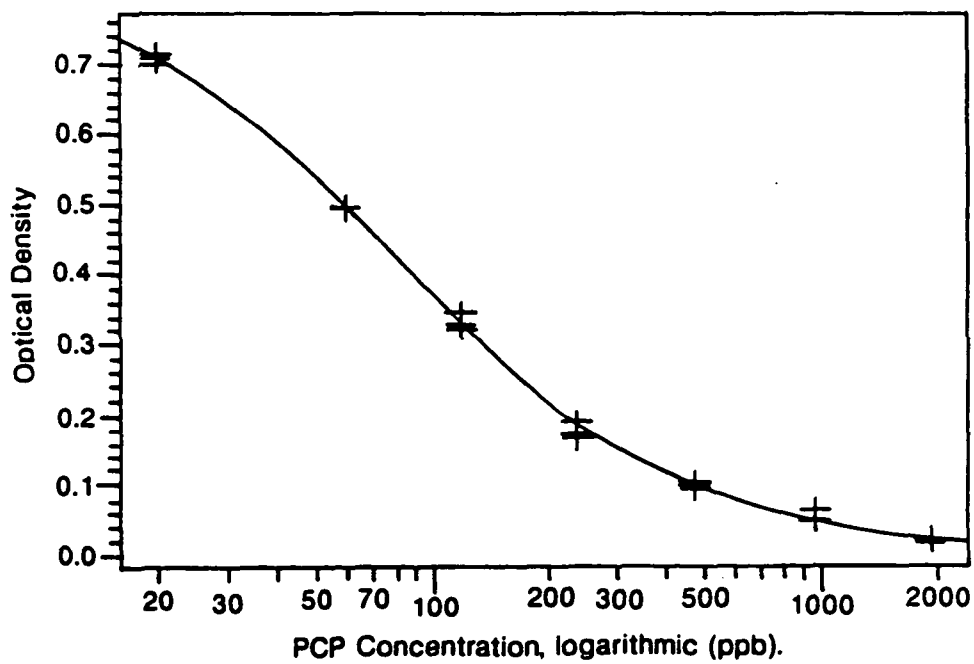
Westinghouse Bio-Analytic Systems Co.
15225 Shady Grove Rd.
Suite 306
Rockville, Maryland 20850

QUALITY CONTROL

1) The minimum acceptable O.D. for the negative control (1 X kit dilution buffer) is 0.50. If the O.D. level falls below this level, the strip should be repeated.

2) Check that the semi-log standard curve (kit standard PCP concentration vs O.D.) for each strip is reasonably linear, by eye. An example of a reasonably linear standard curve is shown below. Check that the O.D. values for dilutions of QA and field samples are in the expected order. If both these criteria are not met, the strip should be repeated.

3) If repeated strips do not meet the QC criteria, call Dr. Jeanette Van Emon at (702) 798-2154, before proceeding with analysis.



APPENDIX III

CALCULATIONS

1) Calculation of percent recovery for spikes of a solution with a standard of known concentration.

   a) Calculate the change in concentration ΔS expected due to the spike.
   b) Measure the concentration of the solution before spiking (C_o) and the concentration of the spiked solution (C_s).
   c) The percent recovery is the percent of the change in concentration relative to the expected change.

   $$\text{percent recovery} = 100\ (C_s - C_o)/\Delta S$$

2) Calculation of the coefficient of variation (%CV). The coefficient of variation is a measure of scatter or dispersion and is defined as the ratio of the standard deviation to the mean.

   $$\%CV = 100(S_x/\bar{x})$$

   where $\bar{x}$ = the mean concentration for a sample of size n.

   S_x = the standard deviation of the mean.

3) Calculation of accuracy, or the degree of agreement of a measurement (or a limiting mean of measurements), x, with an accepted reference or true value, t. Accuracy can be expressed as the difference between the two values, as a percentage of the reference or true value.

   $$\text{Accuracy} = 100\ (x-t)/t$$

APPENDIX B

EPA FACT SHEET:
SITE DEMONSTRATIONS OF TWO TECHNOLOGIES AT THE
MACGILLIS & GIBBS SITE;

MAP OF MACGILLIS & GIBBS SITE LOCATION

EPA Fact Sheet MPCA

SITE Demonstrations of Two Technologies at the MacGillis & Gibbs Site:

1. Soil Washing with the BioTrol Soil Treatment System
2. Biological Treatment of Contaminated Water with the BioTrol Aqueous Treatment System

The MacGillis & Gibbs site has been proposed as a test site for the demonstration of two cleanup technologies under a new U.S. Environmental Protection Agency (EPA) program called the Superfund Innovative Technology Evaluation (SITE) program. The technologies proposed for testing at the MacGillis & Gibbs site were developed by BioTrol, Inc. of Chaska, Minnesota. One of the technologies was designed to treat contaminated soils; the other technology treats contaminated groundwater and wastewater. If approved, the demonstrations will occur in July or August, 1989. The purpose of this Fact Sheet is to provide information on the proposed project and solicit public comment. EPA staff will discuss the project and ask for public comments at the regular meeting of the City of New Brighton Environmental Quality Commission at 7:30 PM, April 12, at New Brighton City Hall, in Council Chamber, 803 Fifth Avenue, NW, New Brighton, Minnesota. The SITE Demonstrations will be the first topic of discussion.

What is the Problem?

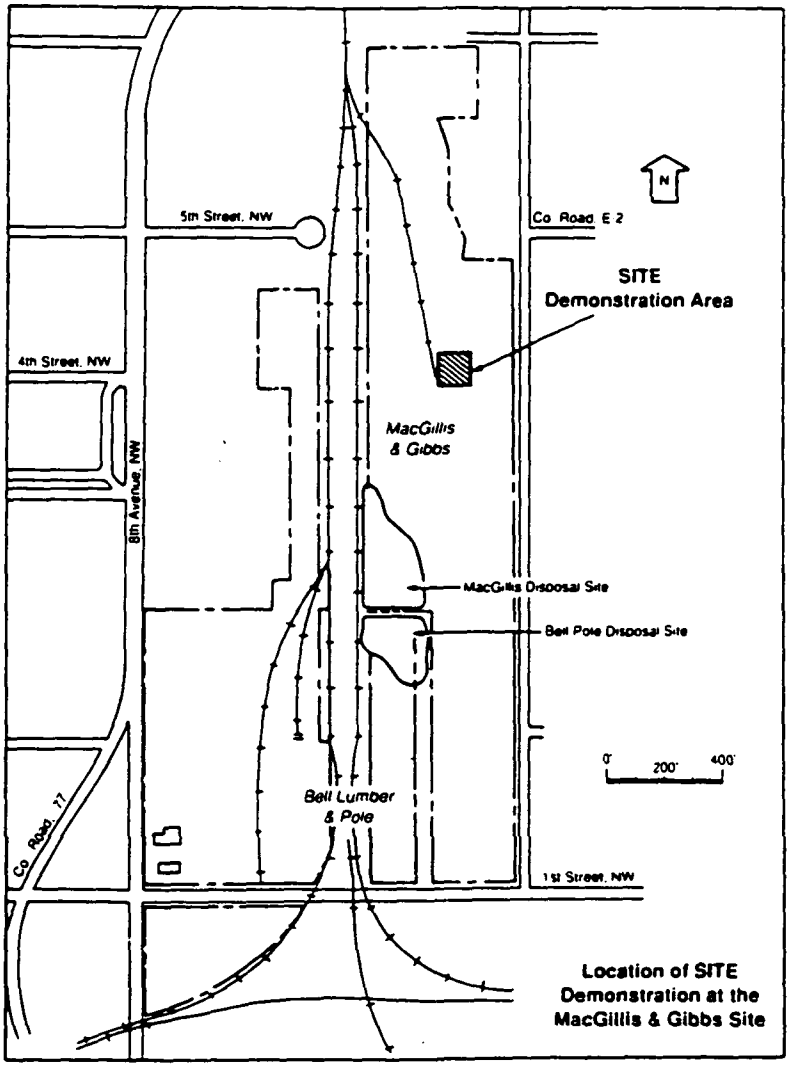
MacGillis & Gibbs, Inc. has been operating a wood treating facility on a 24-acre site in New Brighton, Minnesota since the early 1920's. Originally, a preservative known as creosote was used to treat wood products until the use of pentachlorophenol (penta) was initiated in the late 1940's or early 1950's. In 1970, MacGillis & Gibbs installed a chromated copper arsenate (CCA) pressure treating plant and currently uses only that process. Waste management practices associated with the current wood treating operations conform to current regulations. However, for many years MacGillis & Gibbs and the neighboring Bell Lumber & Pole facility disposed of wastes in a low-lying area astride the properties. The wastes included treated and untreated wood, sludge, and runoff water from the MacGillis & Gibbs treatment area. Studies show that soil throughout the two sites and groundwater under the disposal area are contaminated with the toxic chemicals used in the wood preserving process.

The MacGillis & Gibbs and the Bell Lumber & Pole sites were nominated (as a single 68-acre site) for inclusion on the EPA's National Priority List (NPL) in 1983. In 1984 the site was permanently included on the NPL. Bell Lumber & Pole entered into an agreement with the Minnesota Pollution Control Agency (MPCA) in 1985 to investigate and clean up its portion of the site. The contamination migrating from the MacGillis & Gibbs site is being studied under the auspices of both the EPA and the MPCA Superfund programs.

What is the SITE Program?

The Environmental Protection Agency is trying to find better solutions to hazardous waste cleanup through its new SITE program, which was created in response to the Superfund Amendments and Reauthorization Act of 1986. As a joint effort between EPA's Office of Research and Development and Office of Solid Waste and Emergency Response, the SITE program conducts carefully planned demonstration projects to test new ways to destroy, neutralize, or otherwise detoxify hazardous wastes.

EPA will select suitable locations for SITE demonstration projects after a nationwide search to match promising technologies with the types of wastes and conditions at selected Superfund sites. During the first two years of the SITE program approximately twenty sites across the country were proposed to test various technologies. MacGillis & Gibbs is one of the sites nominated for pilot testing of two innovative treatment technologies.



Science Applications International Corporation (SAIC) is assisting the EPA in evaluating the BioTrol technologies. For the demonstrations at the MacGillis & Gibbs site, SAIC will sample and analyze the materials before and after treatment, and monitor operating parameters such as temperature, flow rates, and power consumption. Finally, SAIC will help the EPA conduct a performance and cost assessment for each demonstration to determine whether the technology is feasible for use at Superfund sites.

What Technologies Will Be Demonstrated at the MacGillis & Gibbs Site?

The EPA is evaluating the BioTrol Soil Treatment System (BSTS) and the BioTrol Aqueous Treatment System (BATS). The BSTS is a volume reduction step for treatment of contaminated soils. During the BSTS process, the larger particles of the soil (the sand) are separated from the smaller soil particles (silt and clay) where the contaminants concentrate. The BATS, a microbiological treatment process for destroying toxic organics, will be tested for cleanup of contaminated groundwater from under the MacGillis & Gibbs site. The BATS will also be used to degrade the toxic organics in the wastewater from the BSTS test. The objective of both treatment technologies is to produce nonhazardous materials for disposal.

Which Contaminants Will Be Treated During the SITE Demonstrations?

The BSTS will be tested on soils contaminated with wood treating chemicals including penta, polynuclear aromatic hydrocarbons (PAHs) present in creosote, and copper, chromium, and arsenic (from the CCA solution). It is expected that the BSTS will remove penta, PAHs, and the CCA metals from the sand portion of the soil. The BATS will be tested on water contaminated with penta and PAHs, both of which are expected to be removed.

Will the Proposed Demonstrations Interfere with the Studies Currently Being Conducted?

The investigations underway as a result of EPA and MPCA Superfund activities will not be delayed or disrupted by the SITE demonstrations. In fact, the SITE data will prove useful in evaluating treatment alternatives for the MacGillis & Gibbs site and selecting the remedial action.

How Does the BioTrol Soils Treatment System Work?

The BSTS operates on the principle that most of the contaminants present at the site are associated with the silt and clay particles and that removal of these particles leaves the rest of the soil (mostly sand particles) relatively clean. Thus, the BSTS is a waste volume reduction technology. It produces a smaller, more easily treated, amount of hazardous waste. First, excavated soils will be passed through a large screen to remove debris. Next, the soil will be mixed with water to form a slurry. The resulting slurry will be screened again and subjected to a series of intensive scrubbing and physical separation steps in a multi-stage washing circuit. The slurry will be separated into a washed sand and a silt and clay slurry containing most of the contamination.

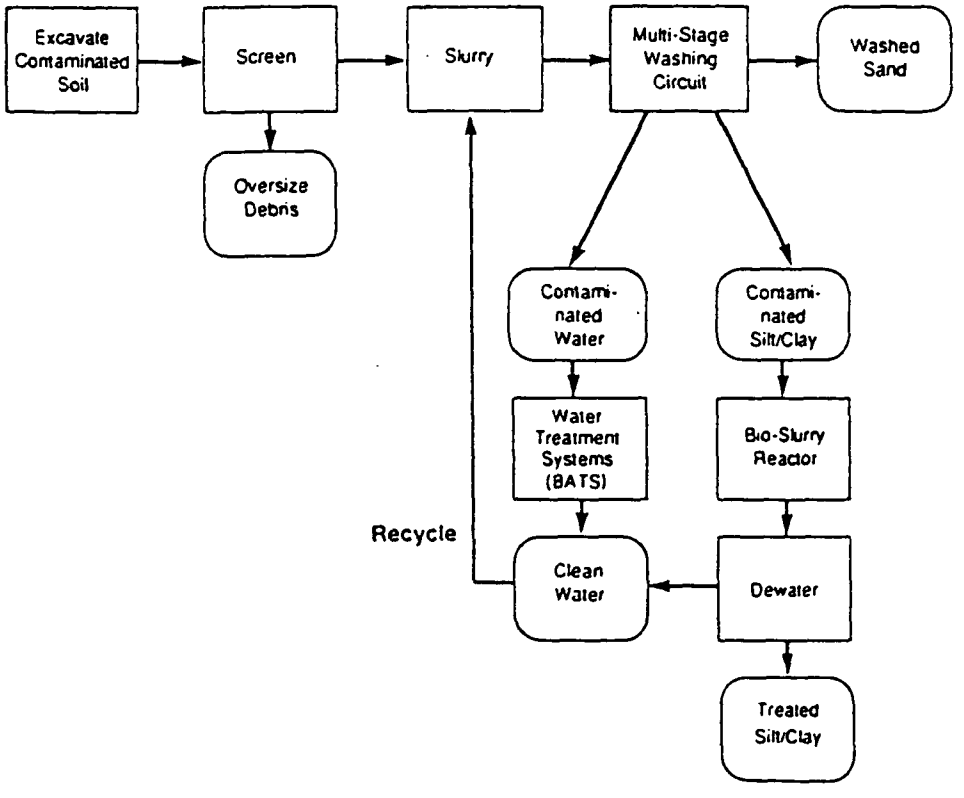
Some of the contaminated silt and clay produced by the BSTS will be further treated biologically by a technology jointly developed by BioTrol and EIMCO Process Equipment Company. The EIMCO Bio-Slurry Reactor (EBSR) will biodegrade the contaminants concentrated in the silt and clay, producing a treated silt and clay.

The BSTS performance will be assessed with soils having two different penta concentrations: about 200-500 ppm, and about 1500-2000 ppm. The system will be tested during continuous 24-hour operation. About 75 tons of contaminated soil will be treated during the 6-8 week SITE

demonstration. Because the EBSR is built on a smaller scale than the BSTS, only part of the silt and clay from the BSTS will be treated. Altogether, the soil treatment tests will produce about 62 tons of washed sand, 18 tons of contaminated silt and clay, 4 tons of washed and biologically treated silt and clay, and 8 tons of wood particles. The increase in total weight of material results from water added during the treatment. The residuals from the treatment system, including the washed sand, the contaminated silt and clay, and the biologically treated silt and clay, will be stored in drums at the site for disposal as part of the Superfund cleanup, or for disposal offsite as a hazardous waste. The washed sand and biologically treated silt and clay may be disposable offsite as nonhazardous waste.

All wastewater produced by the soil treatment system will be treated in the BATS reactor, where contaminants will be broken down by naturally occurring bacteria. If the treated wastewater meets local standards, it will be discharged to the sanitary sewer for treatment and disposal.

During 1988, a pilot test of this technology was conducted at the MacGillis & Gibbs site. The mobile pilot system, treating up to 500 pounds of soil per hour, demonstrated removal of 85 to 99 percent of the penta and PAHs from the contaminated soil. From 73 to 83 percent of the original soil was recovered as washed sand. The favorable results of this pilot test indicate that the proposed SITE demonstration should be successful.



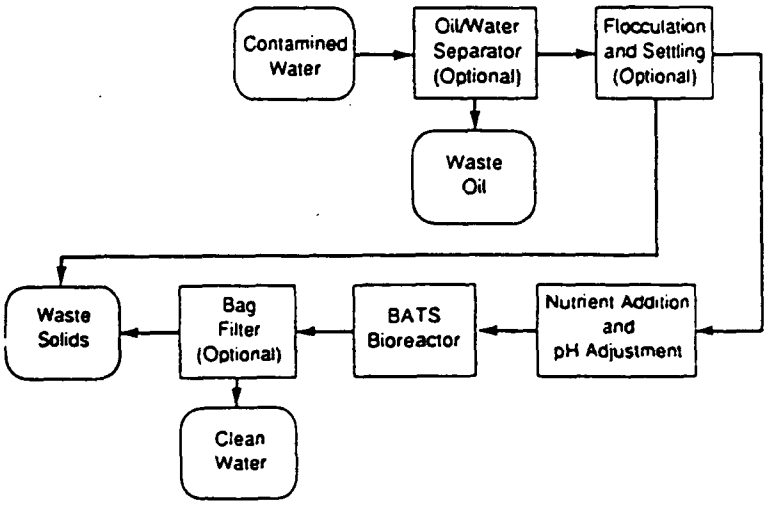
The BioTrol Soil Treatment System (BSTS)

How Does the BioTrol Aqueous Treatment System Work?

The BATS is a microbiological system, consisting of a layer of naturally occurring microbes growing on plastic support material in tanks, used for degrading toxic organic compounds in water. Under the planned SITE demonstration, BioTrol will apply the BATS process to removing the penta and PAHs from the groundwater underlying the MacGillis & Gibbs site. The contaminated groundwater may require pretreatment, such as oil/water separation (to remove floating oil) or flocculation and settling (to remove suspended solids), before passing through the BATS. The pH of the water (a measure of acidity) will be adjusted and inorganic nutrients will be added. These additions help to optimize the performance of the microbes used in the process. In the BATS bioreactor, BioTrol adds a specific naturally occurring microorganism to the microbes which already exist in the groundwater. This combination of microbes rapidly degrades the penta and PAHs into carbon dioxide, water, and inorganic chloride, which are harmless products. A bag filter will be used to capture the excess biomass which exits the bioreactor. This material consists of microbes, both alive and dead, which detach from the supports and are flushed out with the water stream. The bag filter will be replaced periodically. The small amount of residuals from the BATS, including separated oil, flocculated and settled solids, and bag filters containing biomass, will be stored in drums at the site for disposal as part of the Superfund cleanup, or properly disposed of offsite as hazardous wastes.

The SITE demonstration for the BATS will last about 60 days. A maximum of 400,000 gallons of groundwater will be treated during the test. This treated water will be further treated with carbon to remove any remaining contaminants. The water will either be recycled to MacGillis & Gibbs for use in their treatment process or sent to the sanitary sewer for treatment and disposal.

In a nine-month groundwater treatment test conducted at the adjacent Bell Lumber & Pole site from September, 1986 to May, 1987, the BATS process successfully reduced 60-100 ppm levels



The BioTrol Aqueous Treatment System (BATS)

of penta to less than 5 ppm in the treated water. At times, the residual penta was reduced to less than 0.5 ppm. PAH levels of 12 ppm were reduced to 0.5 ppm. The favorable results of this pilot test indicate that the proposed SITE demonstration should be successful.

Have Potential Environmental Effects of the Demonstration Testing Been Evaluated?

Potential effects on air quality, water quality, wetlands and other environmentally sensitive areas, and on threatened or endangered animals or plants have been evaluated. No adverse effects on human health or the environment will be caused by either of the two technologies being demonstrated.

Who Will Be at the Public Meeting?

In accordance with EPA policy, a public meeting has been scheduled for April 12, 1989 at 7:30 PM. Representatives from MPCA, EPA, BioTrol, Inc., and SAIC will be present to explain the proposed SITE demonstrations, and answer any questions that might be raised during the meeting. The general public is invited, including the citizens of New Brighton, environmental groups, and other interested parties.

When is the Public Comment Period?

The MPCA and EPA invite comments on the information presented in this Fact Sheet, as well as any materials discussed during the public meeting. The public comment period will end on May 10, 1989. Written comments should be addressed to:

Minnesota Pollution Control Agency
520 Lafayette Road
St. Paul, MN 55155
Attn: Susan Brustman

Who Can I Contact with Questions about the SITE Demonstrations?

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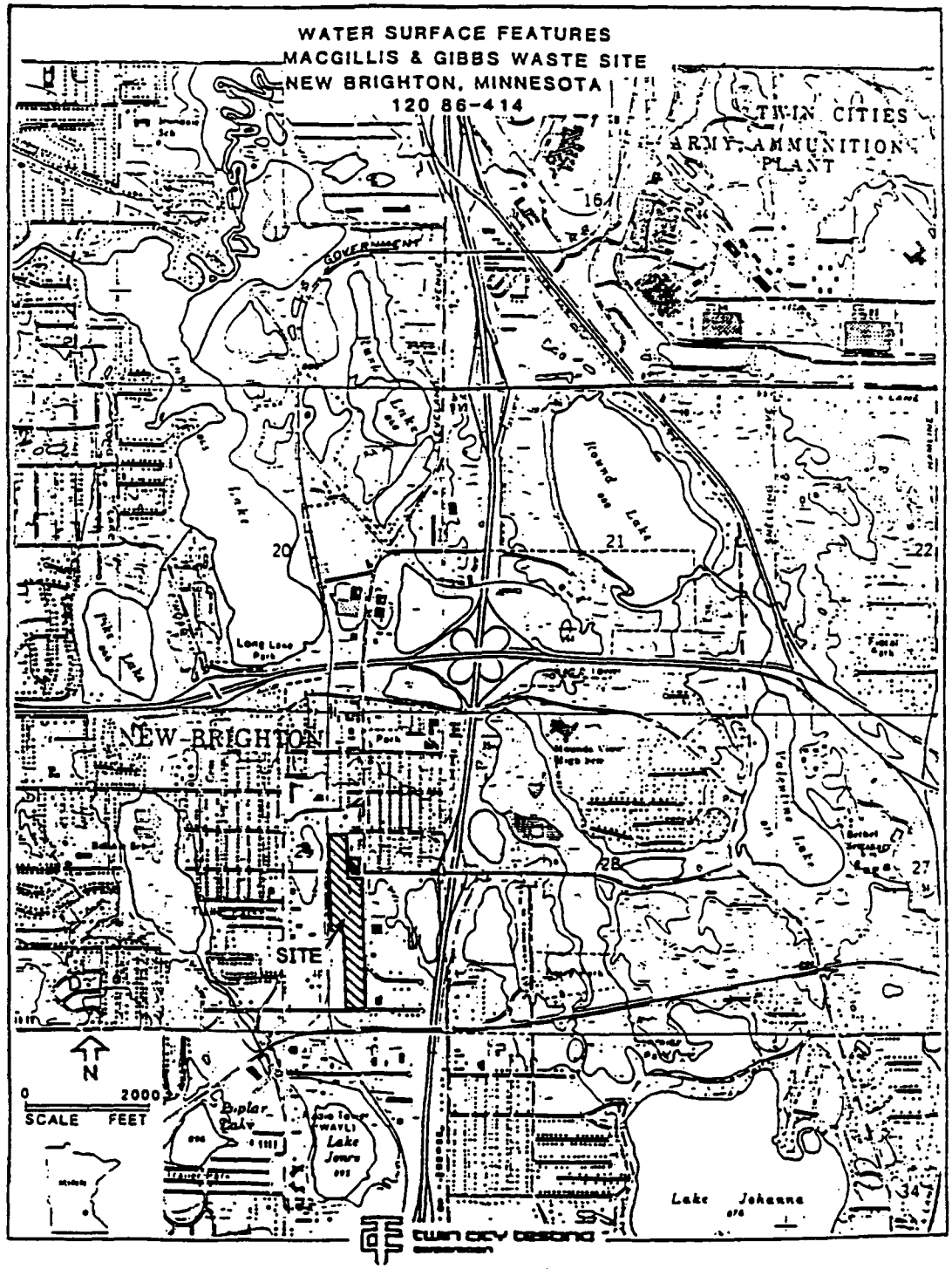

Provided by Twin City Testing Corporation

Figure B-1. MacGillis & Gibbs Site Location Map (site marked by hatched area).

APPENDIX C

STANDARD ANALYTICAL PROCEDURES AND METHODS REFERENCES FOR THE BIOTROL SITE DEMONSTRATION

Source: Draft Demonstration Test Plan for BioTrol, Inc., Biological Treatment of Contaminated Groundwater at New Brighton, Minnesota. 1989. Draft Document, Science Applications International Corporation (SAIC), Paramus, New Jersey, pp. 38-40.

Standard Analytical Procedures and Method References

Measurement	Matrix Type	Method Number	Title	Method Type	Reference[a]
Volatile Organics	Groundwater	SW 8240	Gas Chromatography/Mass Spectrometry for Volatile Organics	Purge and trap/ GC/MS	SW 846
Organic Extraction	Groundwater	SW 3510	Separatory Funnel Liquid-Liquid Extraction	Solvent Liq-Liq extraction	SW 846
Organic Extraction	Solid (Sorbent Resin)	SW 3540	Soxhlet Extraction	Soxhlet Organic Solvent extraction	SW 846
Organic Extraction	Solid (Biosludge)	SW 3550	Sonication Extraction	Solvent extraction by sonication	SW 846
Semivolatile Organics	Solvent Extract	SW 8270	Gas Chromatography/Mass Spec-for Semivolatile Organics: Capillary Column Technique	GC/MS	
Dioxins	Groundwater	SW 8280	The Analysis of Polychlorinated Dibenzo-P-Dioxins and Polychlorinated Dibenzofurans	GC/MS	
Metals Extraction (Cd,Cr,Cu,Pb,Ag, Be,Ni,Zn)	Groundwater	SW 3010	Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by Flame Atomic Absorption Spectroscopy or Inductively Coupled Plasma Spectroscopy	Acid Digestion	
Metals Extraction (As,Sb,Se,Tl)	Groundwater	SW 3020	Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by Furnace Atomic Absorption Spectroscopy	Acid Digestion	

Standard Analytical Procedures and Method References

Measurement	Matrix Type	Method Number	Title	Method Type	Reference[a]
Metals Extraction	Solid	SW 3050	Acid Digestion of Sediment, Sludges and Soils	Acid Digestion	SW 846
Metals - Mercury	Groundwater	SW 7470	Mercury in Liquid Waste (Manual Cold Vapor Technique)	Cold Vapor - AA	SW 846
Metals - Mercury	Solid	SW 7471	Mercury in Solid or Semisolid Waste (Manual Cold Vapor Technique)		SW 846
Metals Analysis (Cd,Cr,Cu,Pb,Ag, Be,Ni,Zn)	Groundwater - Digestate	SW 6010	Industively Coupled Plasma Atomic Emission Spectroscopy	ICP	SW 846
Metals Analysis:	Groundwater -				
Arsenic	Digestate	SW 7060	Arsenic (AA, Furnace Technique)	GFAA	SW 846
Antimony		SW 7041	Antimony (AA, Furnace Technique)	GFAA	SW 846
Selenium		SW 7740	Selenium (AA, Furnace Technique)	GFAA	SW 846
Thallium		SW 7841	Thallium (AA, Furnace Technique)	GFAA	SW 846
Chloride	Groundwater	SW 9252	Chloride (Titrimetric, Mercuric Nitrate)	Titrimetric	SW 846
Total Organic Carbon (TOC)	Groundwater	SW 9060	Total Organic Carbon	Combustion/IR Detector	SW 846
Phenolics	Groundwater	SW 9065	Phenolics (Spectrophotometric Manual 4AAP with Distillation)	Colorimetric/ Spectrophotometric	SW 846
Oil and Grease	Groundwater	SW 9070	Total Recoverable Oil and Grease (Gravimetric, Separating Funnel Extraction)	Solvent Extraction/ Gravimetric	

Standard Analytical Procedures and Method References

Measurement	Matrix Type	Method Number	Title	Method Type	Reference[a]
Residue	Groundwater	SM 209	Residue	Gravimetric	SM
Alkalinity	Groundwater	SM 403	Alkalinity	Titrimetric	SM
Nutrients:					
Ammonia	Groundwater	SM 417	Nitrogen (Ammonia)		SM
Nitrate	Groundwater	SM 418	Nitrogen (Nitrate)		SM
Phosphate	Groundwater	SM 424	Phosphorous		SM
Field Measurements:					
pH	Groundwater	SM 423	pH Value	Direct Measurement	SM
Dissolved Oxygen	Groundwater	SM 421	Oxygen (Dissolved)	Direct Measurement	SM
Temperature	Groundwater	SM 212	Temperature	Direct Measurement	SM

[a]SW 846 - "Test Methods fore Evaluating Solid Waste", SW 846, Third Edition, November, 1986
SM - "Standard Methods for the Examination of Water and Wastewater" 15th Edition, 1980.

APPENDIX D

SAMPLE SPLITTING AND SHIPPING INSTRUCTIONS FOR THE IMMUNOASSAY SITE DEMONSTRATION

SAMPLE SPLITTING AND SHIPPING INSTRUCTIONS FOR THE IMMUNOASSAY SITE DEMONSTRATION

During each of the three 6-day spans comprising the formal demonstration, splits of every field sample analyzed by the field immunoassay kit (all routine influents, routine effluents, field blanks, field duplicates, raw influent grabs) are to be sent to both the EMSL-LV and WBAS laboratories. About 500-600 mL is split from each well mixed field sample for immunoassay analysis. Two large glass bottles are filled with 250-300 mL from each sample. The bottles are labelled with an SAIC label, capped with a teflon-lined screw cap, packed in ice and shipping insulation in a shipping cooler and shipped to the addresses below. For field analysis, 20-30 mL of each sample* is placed in a small amber glass bottle, labelled with an SAIC label (as above). The bottles are capped tightly and stored at 4 °C on-site. A small volume is used for one-site field kit PCP analysis, and the remainder is archived until all the analyses for the project are completed.

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For GC/MS Analysis at EMSL-LV on a few selected samples, the following sampling and shipping procedure is to be used. On one day of each of the six 6-day spans (see below) an additional 1 liter of the routine (daily) influent, effluent and water blank are split off and placed in a large (1 liter) amber glass bottle. In addition, once during the six weeks comprising the bioreactor demonstration, a 1-liter split of the raw influent grab (selected for SAIC GC/MS analysis) sample is to be taken and bottled. The bottles are labelled as above, capped tightly with a teflon-lined screw cap, and carefully packed in ice and shipping insulation in a shipping cooler. The samples are sent to the above EMSL-LV/LESC address for confirmatory GC/MS analysis.

*The small volume splits of the routine effluent samples made for field analysis and archiving are to be centrifuged prior to bottling.

APPENDIX E

LIST OF ADDRESSES FOR SHIPPING BIOREACTOR AND QA/QC SAMPLES FOR THE IMMUNOASSAY SITE DEMONSTRATION

FIELD SITE ADDRESS (FIELD IA)

MacGillis and Gibbs
440 5th Ave. N.W.
New Brighton, Minnesota 55112

Attn: Randy Potter

ADDRESS FOR SAIC (GC/MS)

Sample Custodian
SAIC
4224 Campus Point Court
Mailstop 210
San Diego, California 92121

ADDRESS FOR WBAS (LAB & FIELD IA)

Westinghouse Bio-Analytic Systems
15225 Shady Grove Rd.
Suite 306
Rockville, Maryland 20850

Attn: Dr. Steve Soileau

QA SAMPLE SHIPMENT

FIELD SITE

No.		Total Volume	Analysis Type
16	Type A QA Amps (2 mL) each with 0.5 mL	8 mL	Field IA
8	Type B QA Amps (2 mL) each with 0.5 mL	4 mL	Field IA
6	20 ppm QC Performance Sample Amps (5 mL), each with 2 mL	12 mL	Field IA
6	15 ppm I.S. Spike Std. Amps (2 mL), each, with 1 mL	6 mL	Field IA

WESTINGHOUSE BIO-ANALYTIC SYSTEMS

No.		Total Volume	Analysis Type
14	Type A QA Amps (5 mL) each with 2 mL	28 mL	Lab IA
8	Type B QA Amps (5 mL) each with 2 mL	16 mL	Lab IA
14	Type A QA Amps (2 mL) each with 0.5 mL	7 mL	Field IA
8	Type B QA Amps (2 mL) each with 0.5 mL	2 mL	Field IA
6	20 ppm QC Performance Sample Amps (5 mL), each with 2 mL	12 mL	Field IA
6	15 ppm I.S. Spike Std. Amps (2 mL), each, with 1 mL	6 mL	Field IA

SCIENCE APPLICATIONS INTERNATIONAL CORP

No.		Total Volume	Analysis Type
12	Type A QA Amps (5 mL) each with 5 mL	60 mL	GC/MS
6	Type B QA Amps (5 mL) each with 5 mL	30 mL	GC/MS
6	Type A QA Amps (2 mL) each with 2 mL	12 mL	GC/MS
3	Type B QA Amps (2 mL) each with 2 mL	6 mL	GC/MS

IA = immunoassay

Amps = Ampules

IS = internal standard

APPENDIX F

INSTRUCTIONS FOR COMPLETING THE FIELD DATA FORM FOR THE FIELD KIT ANALYSIS

INSTRUCTIONS FOR FILLING OUT THE FORM ENTITLED "FIELD DATA FOR ANALYSIS OF WBAS FIELD IMMUNOASSAY TEST KIT"

GENERAL

1) A field form is to be filled out for each sample analyzed. For instance, when a daily influent (RI) and a daily effluent (RE) sample are analyzed on the same day, a field form will be completed for each sample.

2) The filling out of page numbers (top right corner of each form) should be done after all the analyses for one sample are completed. "Page 1 of _" will, by definition, be the first page (with the sample collection, environmental factors, and sample specific information included along with the results of the analysis of the first strip for the sample). "Page _ of _" is designed to accommodate all subsequent strips (i.e., range-finding, duplicate/split, matrix spike, and/or QA and QC strips) needed to complete the analysis of a given sample on a given day.

3) The circles located after many of variables on the form are to indicate that a qualifying comment (tag) relates to that particular piece of information. Place an 'X' or 'Y' or some other tag in (or legibly over) the circle and then "footnote" it in the 'Comments' section. This should direct the data reviewers to issues relating to that particular variable.

4) Use a ball point pen with ink that will not run. Apply sufficient pressure to ensure that all carbonless sheets are legible. When illegible or incorrect entries are made on the form, use a single line to cross out; then initial the line and put correct information immediately next to it if there is room (if necessary, note correct information in the 'Comments').

5) After reviewing all the entries on the completed forms:

- o send the top (WHITE) copy to Las Vegas via overnight courier along with the printout (cash register tape) for the data from that sample
- o send the middle (CANARY) copy to WBAS via overnight courier
- o retain the bottom (PINK) copy on file on site

NOTE: If logistically possible, send field forms with corresponding field samples or samples from the next shipping day; when sending forms in boxes with field samples, use plastic Ziploc bags.

The specific instruction for entering field data on the forms are discussed below by "INFORMATION BLOCK" and by 'Variable' as they appear on the forms.

SAMPLE COLLECTION INFORMATION

(NOTE: This block contains the SAIC sample bottle label data and other information)

Sample Date: enter in the day, month, year in the following format: '23JUL89' or '04AUG89'.

Sample Time: in military time.

SAIC Sample No.: enter the sample number as described in the sampling plan (e.g., 'ST1-A-01-01' is an 'influent sample' taken on the 'first day' of flow rate period 'A' during 'Stage 1').

Collection Method: circle one, usually 'Composite'.

ISCO ID: identification number noted on the sampling device.

Preservative: usually '4°C'.

Bioreactor ID: BATS identification number.

Groundwater Well No.: as applicable to MacGillis & Gibbs Site.

Flow Rate: usually either '1' or '3' or '5' gallons per minute.

BATS PCP Spike: 'yes' or 'no' (yes, for Stage 2 of the BATS demonstration).

BATS Spike Conc: concentration (ppm) of PCP spike (added to the influent in Stage 2).

Collected by: initials of field crew member who collected the sample.

Split by: initials of field crew member who prepared the splits of the composite for on-site and off-site analysis.

Comments: any comments applicable to the information in this block, and any other information related to the sample collection or handling.

ENVIRONMENTAL FACTORS

Analysis Date: the date the sample is analyzed, in same format as Sample Date (e.g., '23JUL89').

Days Since Collected: number of calendar days between sampling and analysis (NOTE: if 'Sample Date' and 'Analysis Date' are the same, enter '00').

Site of Analysis: circle applicable site.

Location of Analysis: circle one, usually 'Indoors'.

Location Temp.: ambient temperature where analysis performed.

Sunny/Cloudy/Mixed/, Windy/Calm, Other: applicable to outdoor factors, if sample analyzed there.

Kit Lot No.: lot number on the WBAS kit box.

Kit Storage Days: number of days kit was stored at site location (both in and out of the refrigerator), from date of receipt to date of analysis.

Daily Refrig. Temp.: refrigerator temperature at the time the kit is removed for analysis activities.

Comments: any pertinent comments related to the information in this block.

Analyst: initials of the crew member performing the test kit analysis.

SAMPLE-SPECIFIC PRE-ANALYSIS INFORMATION

Sampling Point Source: as applicable.

Sample Appearance: as applicable for color, clarity, presence of precipitate (or settleable matter) or oil, etc.

Centrifuged: usually for effluent samples to remove biomass, etc.

Appearance Change: applicable only if there are observed differences in sample appearance after centrifugation.

pH-Meter: result of on-site sample analysis performed by pH meter.

pH-Paper: result of on-site sample analysis performed by pH paper (NOTE: this may be optional).

Hardness-Paper: result of on-site sample analysis by hardness paper (NOTE: this may be optional).

Field Blank: indicate if a field equipment wash blank sample was collected with this sample and the applicable SAIC Sample No. Any pertinent pre-analysis information about this blank should be noted in the 'Comments'.

Field Duplicate: indicate if a field duplicate sample was collected with this sample and if there is an SAIC Sample No. Any pertinent pre-analysis information about this duplicate sample should be noted in the 'Comments'.

Field QA Audit: indicate if a QA audit sample, type A or B was analyzed with this sample (usually with a routine effluent sample on a "long day"; see sampling schedule and protocols). Include the LESC Sample No. (e.g., 'QAA-022', 'QAB-004') obtained from the ampule label.

Comments: any comments related to the information in this block.

SAMPLE ANALYSIS INFORMATION BY STRIP

Strip ID: this is a strip identifier comprised of one letter and one number. The letters represent:

A = daily QC strip
B = QA strip
C = effluent range-finding strip
D = influent range-finding strip
E = effluent duplicate/split strip
F = influent duplicate/split strip
G = effluent matrix spike strip
H = influent matrix spike strip
I = cross-calibration strip for field photometer
J to Z = as necessary, only when above letters don't apply

The number after the letter represents how many runs of a particular strip type (A-Z) were done for that sample. Example #1: the daily QC 'Strip ID' is labelled 'A-1'; if a second QC strip must be analyzed because of "bad" QC data (see performance specifications), it will be labelled 'A-2'. Example #2: If the first range-finding strip for a daily effluent ('Strip ID': 'C-1') or influent ('Strip ID': 'D-1') sample yields inadequate dilution concentrations, a second strip with a different dilution scheme will be analyzed (labelled 'C-2' and 'D-2', respectively).

Sample Code: note the applicable sample code for each well, as they appear and are defined on the sample code list (e.g., 'FB', 'QC', 'RE', 'RIMS', 'SDI').

Well#: these will usually be numbered 1 - 8.

OD(ABS): this is the optical density (OD) for each sample as it appears on the LCD readout of the Dynatech photometer and is expressed in absorbance units (ABS). Report the ABS value to three places after the decimal point in this column.
NOTE: when plotting these points on the semi-log graph below, round to two places after the decimal point, because of the limited resolution on the graph.

Plotted Conc. (ppb): the PCP concentration of samples analyzed in the range of the calibration curve (the curve is determined by plotting the absorbance units of the four kit standards against their known (3.0, 7.1, 16.9, 40.0) concentrations and then fitting (estimating by eye) the best straight line between those four points. All sample absorbance units are then plotted on that line so that the concentration, in ppb, can be determined.
NOTE: estimate the ppb concentration to the nearest tenth, if possible (e.g., 8.4 ppb); it is recognized that this will be difficult for samples >10 ppb.

to ppm: multiplying 'Plotted Conc (ppb)' by 0.001 converts ppb to ppm. NOTE: This is <u>not necessary for kit standards</u>.

Dilution Factor: this is a multiplication factor through which samples diluted to fall within the linear dynamic range of the calibration curve are converted to concentrations in their undiluted state. For instance, if a sample is diluted 1:1000, the dilution factor is the inverse, or 1000. (NOTE: if a sample is not diluted or is a kit standard, there is <u>no dilution factor</u>).

Sample Conc (ppm): the result of multiplying:

'Plotted Conc (ppb)' X '0.001' X 'Dilution factor' (if any)
The product should equal the PCP concentration (in ppm) of the sample
(NOTE: this is <u>not</u> applicable to the kit standards).

Amt(ppb) Spike Added: this is <u>only</u> used for MSE or MSI samples and is the concentration (in ppb) of the internal standard (matrix) spike into a routine (REMS, RIMS) sample diluted into specified linear range (~3-20 ppb) for spiking.

% Spike Rec: this is the percent spike recovery and is determined by calculating:

$$\frac{\text{(sample concentration + spike) - unspiked sample concentration}}{\text{concentration of spike added}} \times 100$$

specifically,

$$\frac{\text{MSE - REMS}}{\text{Amt Spike Added}} \times 100 \quad \text{or} \quad \frac{\text{MSI - RIMS}}{\text{Amt Spike Added}} \times 100$$

(NOTE: numerator values are from the 'Plotted Conc (ppb)' column)

Semi-Log Graph: as presented, the X-axis is prelabelled for PCP concentrations in parts per billion ([PCP] ppb) from 2-60 ppb in log scale. The Y-axis is labelled for the optical density (OD) in absorbance units (ABS), but <u>no</u> prelabelled units are given. The Y-axis is <u>not</u> in log scale and is left

to the discretion of the analyst to label. This will depend upon the ABS readings generated from the calibration kit standards analyzed on each strip. For instance, the ABS increments may be labelled:

0.20, 0.40, 0.60, 0.80, 1.00, 1.20;
or 0.30, 0.50, 0.70, 0.90, 1.10; 1.30;
or 0.30, 0.40, 0.50, 0.60, 0.70, 0.80;
or some other increments that 1) cover the absorbance range of the kit standards and, 2) provide maximum resolution (using units that are "reasonable" and easily plotted).

Comments: Any comments that are related to the plotting, diluting, calculating ppms or spike recoveries, or other pertinent information related to sample analysis should be entered in the space provided. The 'tag circle' provided after the 'Sample Code' can be used to denote any sample requiring discussion here. (NOTE: It is recognized that the space provided may be awkward to write in, so writing "sideways" is acceptable.

Page 2, and all other subsequent pages needed for the analysis of a sample, should include the 'SAIC Sample No.' in the "SAMPLE COLLECTION INFORMATION" block, and the 'Analysis Date' and 'Analyst' initials that appears in the "ENVIRONMENTAL FACTORS" block on Page 1. All "SAMPLE ANALYSIS INFORMATION BY STRIP" should follow the same format as described above.

APPENDIX G

DESCRIPTIONS AND SCHEMATICS FOR DAILY SAMPLE ANALYSIS

SAMPLING AND ANALYSIS SCHEDULE
FOR THE FIELD PCP IMMUNOASSAY KIT SITE DEMONSTRATION

PRELIMINARY EVALUATION (bioreactor start-up) 26 days, June 26-July 21.
PART A-(bioreactor flow rate= 1 gal/min) 6 days, July 24-29
PART B-(bioreactor flow rate= 3 gal/min) 6 days, August 7-12
PART C-(bioreactor flow rate= 5 gal/min) 6 days, August 21-26

Use the same sampling and analysis scheme for all three parts. Effluent samples are from reactor 3 (final), unless otherwise stated. The samples referred to as the routine influent or effluent is a split of the composite sample taken for GC/MS analysis, **for that day.**

The routine influent will be taken from the holding tank, containing treated (nutrients added, pH adjusted to 7.2) well water. In addition, once a week, a grab sample of raw or untreated well water will be taken for GC/MS analysis. A split of this grab sample is to be analyzed by the field PCP immonoassay method also. If possible, do the analysis schemes in the order listed: DAY 1 first, DAY 2 second, and DAY 3 third. The short day routines are for days when the person doing the field analysis is busy with other activities and the long day routines are for days when there is more time available. The 4 kit standards that are run on each strip are the following: 3 ppb, 7.1 ppb, 16.9 ppb, and 40 ppb. Each day, run the QC STRIP first.

All samples should be run as soon as possible after collection. Try to run all samples within a 24 hr period after splitting. Try to complete the analysis of all the scheduled samples (see the weekly totals list below) within each of the three 6 day periods. A minimum volume of 20 ml from each field sample analyzed should be placed in a 30 ml amber vial, labelled with an SAIC label (see example below) and capped tightly with a teflon lined screw cap. All these vials are to be stored at 4 degees C, in case later analysis is necessary. If time does not permit the analysis of all the above samples during the 6 day week, unanalyzed samples are to be run on the following week.

QC STRIP

LONG DAY 1 1 strip with a **field water blank**, undiluted and DAY 1 diluted 1 to 10, a negative **control** (1X kit dilution buffer) and a 20 ppm **performance standard**, diluted 1 to 1000 + 4 kit standards.

EFFLUENT STRIPS

1-2 strips with four semi-log serial dilutions (suggested dilutions: 1 to 10, 1 to 50, 1 to 100, and 1 to 500) of the routine effluent + 4 kit standards. 1 strip with the routine effluent diluted to about 10 ppb (3-20 ppb) run in duplicate with and without a 15ppb internal standard spike + 4 kit standards.

QA SAMPLE STRIPS

2 strips with three serial two-fold dilutions of a semi-blind (see instructions) type A QA audit sample + 4 kit standards, and one negative control.

INFLUENT STRIP
1 strip with routine influent at 4 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, 1 to 4000 and 1 to 8000) + 4 kit standards.

LONG DAY 2 - SAME AS LONG DAY 1 .

LONG DAY 3 - SAME AS LONG DAY 1, EXCEPT RUN **TYPE B QA AUDIT SAMPLE.**

QC STRIP

SHORT DAY 1 1 strip with a **field water blank**, undiluted and 1 to 10 diluted, a **negative control** (1X kit dilution buffer) and a 20 ppm **performance standard**, diluted 1 to 1000 + 4 kit standards.

INFLUENT STRIPS
2 strips with routine influent at 3 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, and 1 to 4000) + 4 kit standards, and one **negative control.**

2 strips, with duplicates of both the routine influent and its field duplicate (split), each diluted to near the midpoint (20+/- 10 ppb) of the standard curve + 4 kit standards.

1 strip with split of the routine influent diluted to about 10 ppb (3-20 ppb) and run in duplicate with and without a 15 ppb internal standard spike + 4 kit standards.

QC STRIP

SHORT DAY 2 1 strip with **field water blank**, undiluted and diluted 1 to 10, 1 **negative control** (1X kit dilution buffer) and a 20 ppm **performance standard**, diluted 1 to 1000.

EFFLUENT STRIPS
2 strips each with a split of the routine effluent at 4 dilutions (suggested dilutions: 1 to 10, 1 to 100, to 50 and 1 to 500) + 4 kit standards.

2 strips, with duplicates of both the routine effluent and its field duplicate split, each diluted to near the midpoint of the standard curve (20+/-10 ppb) + 4 kit standards.

INFLUENT STRIP
1 strip with the routine influent at three dilutions (suggested dilutions: 1 to 1000, 1 to 2000, and 1 to 4000) + 4 kit standards and one **negative control.**

QC STRIP

SHORT DAY 3 1 strip with a **field water blank**, undiluted and diluted 1 **to 10, a negative control** (1X kit dilution buffer) and a 20 ppm **performance standard** diluted 1 to 1000 + 4 kit standards.

INFLUENT STRIPS
2 strips with split of the routine influent at 3 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, 1 to 4000) + 4 kit standards, and one **negative control.**

1 strip with split of the routine influent diluted to about 10 ppb (3-20 ppb) and run in duplicate with and without a 15 ppb internal standard spike + 4 kit standards.

DAY FOR INFLUENT GRAB - After running the other strips scheduled for this day, run the following.

INFLUENT STRIPS
2 strips with split of the raw influent grab sample at 3 dilutions (suggested dilutions: 1 to 1000, 1 to 2000, 1 to 4000) + 4 kit standards, and one **negative control.**

WEEKLY TOTALS

1) 6 routine (daily) influent samples

2) 4 routine (daily) effluent samples

3) 1 field duplicate split of a routine (daily) effluent

4) 1 field duplicate split of a routine (daily) influent

5) 1 raw (untreated) influent grab sample

6) 2 type A QA audit samples

7) 1 type B QA audit sample

8) 6 routine (daily) field water blanks (equipment rinse)

9) 6 daily replicates of the 20 ppm Q C performance standard, diluted fresh each day.

10) A minimum of 18 negative controls (1 X dilution buffer from kit)

Proposed Sample Coding Scheme for WBAS Field Immunoassay Demonstration

FB = field equipment blank (after daily decontamination of ISCO and before daily compositing)

NC = negative control sample - dilution/reagent blank water from 1X kit dilution buffer solution

QC = daily quality control performance check sample @ 20 ppm (known concentration)

STD03 = calibration kit standard @ 3.0 ppb (0.0030 ppm) pentachlorophenol

STD07 = calibration kit standard @ 7.1 ppb (0.0071 ppm) pentachlorophenol

STD16 = calibtation kit standard @ 16.9 ppb (0.0169 ppm) pentachlorophenol

STD40 = calibration kit standard @ 40.0 ppb (0.0400 ppm) pentachlorophenol

RE = routine daily effluent sample

RI = routine daily influent sample

DE = duplicate of a raw, undiluted RE sample (i.e., split before any dilutions)

DI = duplicate of a raw, undiluted RI sample (i.e., split before any dilutions)

SRE = split of an RE sample after the RE has been diluted into calibration range

SRI = split of an RI sample after the RI has been diluted into calibration range

SDE = split of a DE sample after the DE has been diluted into calibration range

SDI = split of a DI sample after the DI has been diluted into calibration range

REMS-x = RE sample diluted to the range for matrix spiking which will be used in the % recovery calculation (x = sample to be matched with the same "x" for the MSE)

RIMS-x = RI sample diluted to the range for matrix spiking which will be used in the % recovery calculation (x = sample to be matched with the same "x" for the MSI)

MSE-x = matrix spike sample of a REMS with corresponding "x"

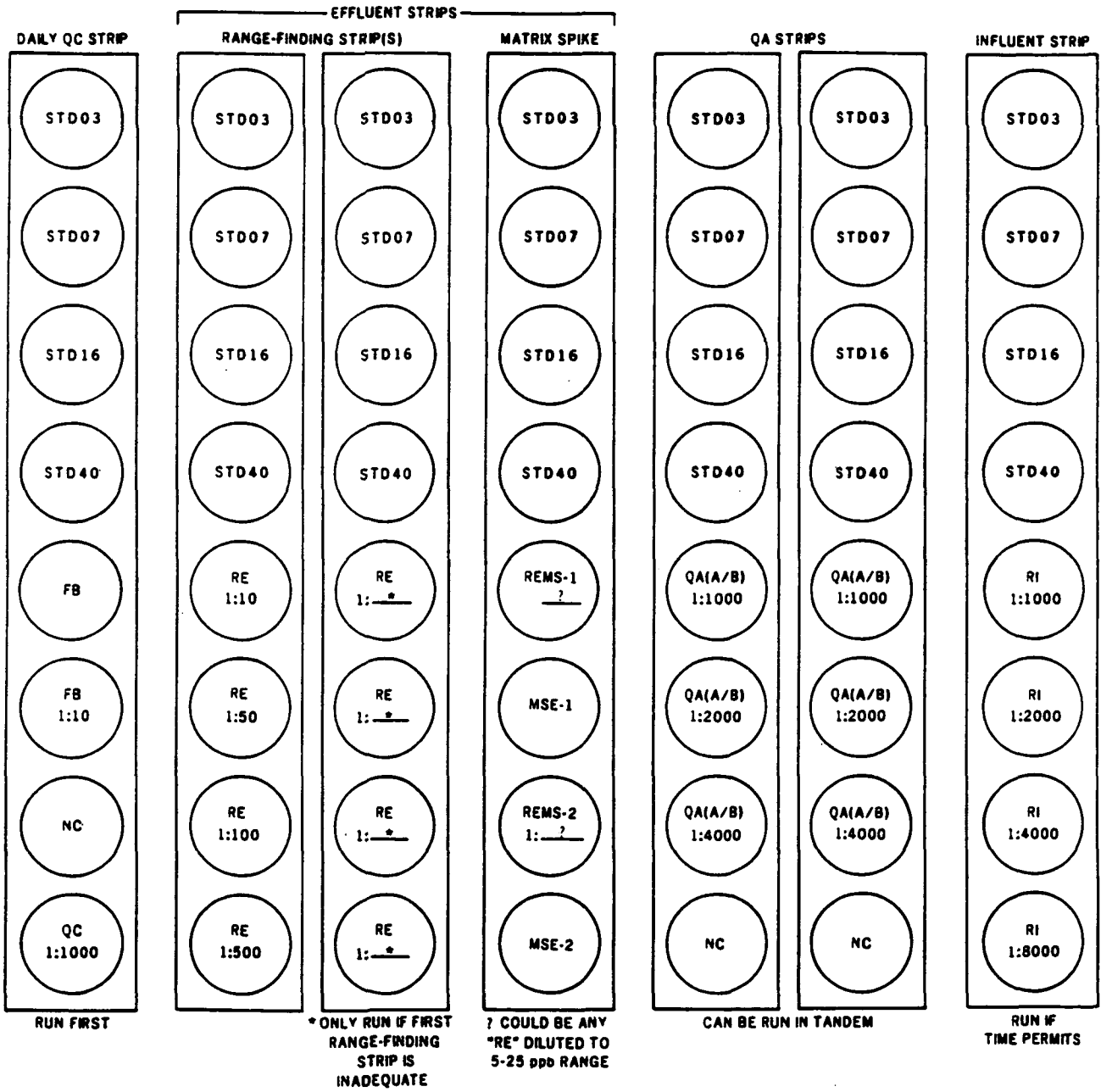
MSI-x = matrix spike sample of a RIMS with corresponding "x"

QAA-xxx = semi-blind QA standard, Lot A (xxx = LESC sample control #)

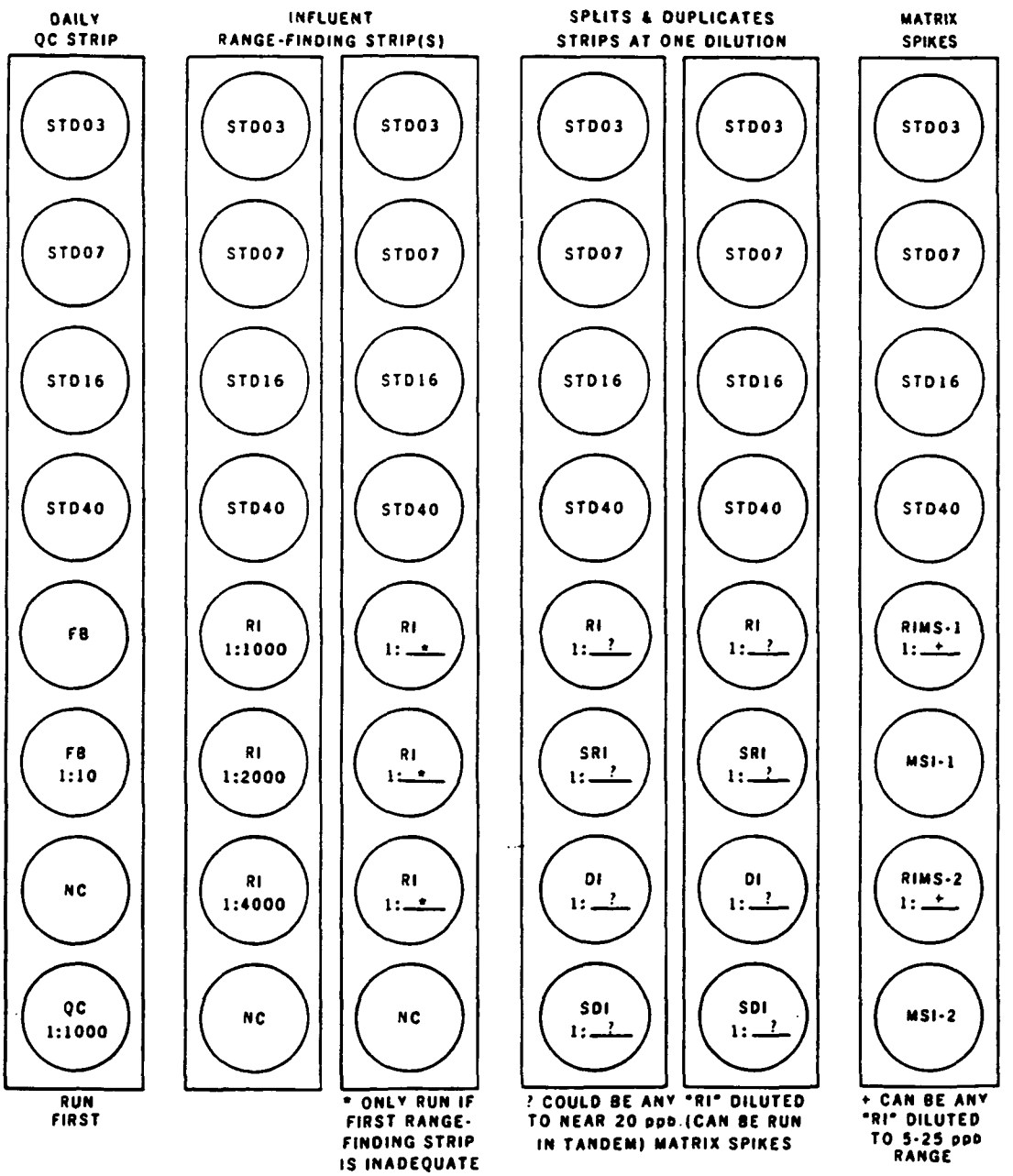
QAB-xxx = semi-blind QA standard, Lot B (xx = LESC sample control #)

CC-xxx = cross-calibration color check standard for photometers (xxx = code for theoretical optical density)

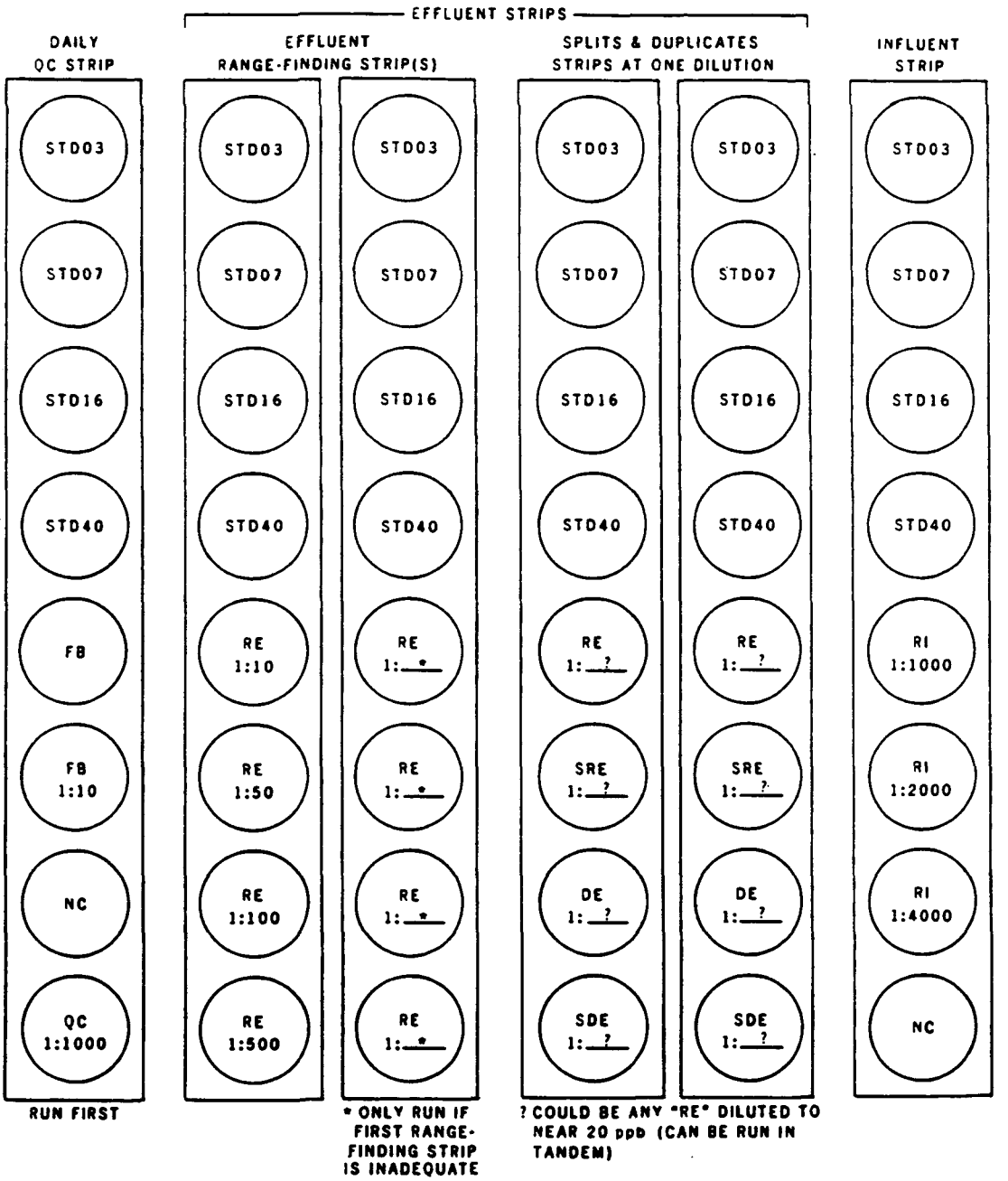
LONG DAYS 1, 2, 3



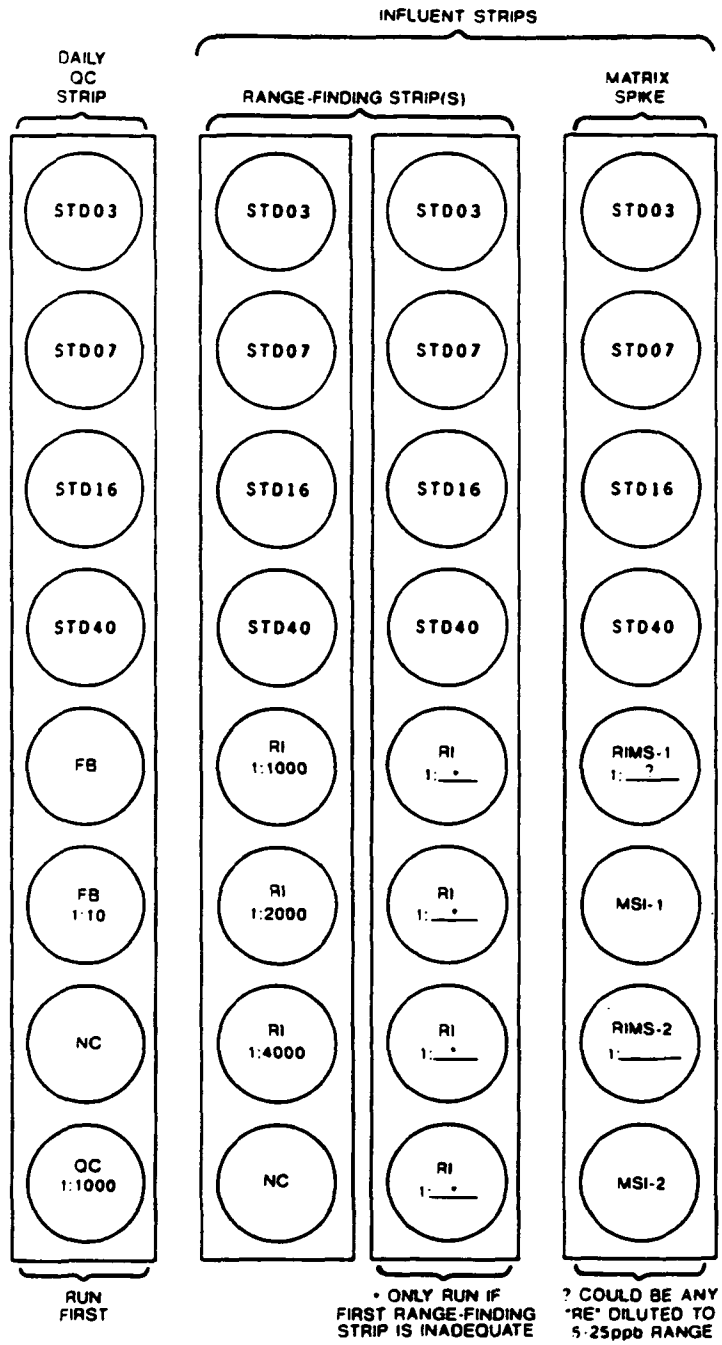
SHORT DAY 1


DAILY QC STRIP
STD03
STD07
STD16
STD40
FB
FB 1:10
NC
QC 1:1000
RUN FIRST
INFLUENT RANGE-FINDING STRIP(S)
STD03
STD07
STD16
STD40
RI 1:1000
RI 1:2000
RI 1:4000
NC
STD03
STD07
STD16
STD40
RI 1: *
RI 1: *
RI 1: *
NC
* ONLY RUN IF FIRST RANGE-FINDING STRIP IS INADEQUATE
SPLITS & DUPLICATES STRIPS AT ONE DILUTION
STD03
STD07
STD16
STD40
RI 1: ?
SRI 1: ?
DI 1: ?
SDI 1: ?
STD03
STD07
STD16
STD40
RI 1: ?
SRI 1: ?
DI 1: ?
SDI 1: ?
? COULD BE ANY "RI" DILUTED TO NEAR 20 ppb (CAN BE RUN IN TANDEM) MATRIX SPIKES
MATRIX SPIKES
STD03
STD07
STD16
STD40
RIMS-1 1: +
MSI-1
RIMS-2 1: +
MSI-2
+ CAN BE ANY "RI" DILUTED TO 5-25 ppb RANGE

SHORT DAY 2

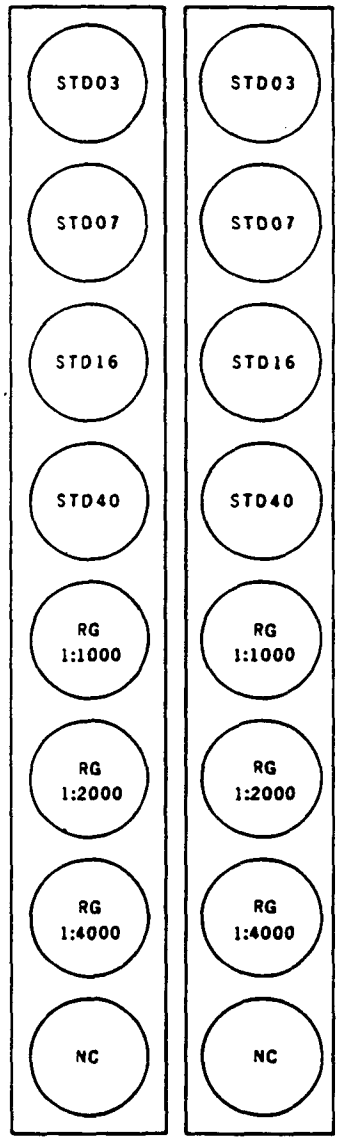

EFFLUENT STRIPS
DAILY QC STRIP
EFFLUENT RANGE-FINDING STRIP(S)
SPLITS & DUPLICATES STRIPS AT ONE DILUTION
INFLUENT STRIP
STD03
STD07
STD16
STD40
FB
FB 1:10
NC
QC 1:1000
RE 1:10
RE 1:50
RE 1:100
RE 1:500
RE 1:*
SRE 1:?
DE 1:?
SDE 1:?
RI 1:1000
RI 1:2000
RI 1:4000
RUN FIRST
* ONLY RUN IF FIRST RANGE-FINDING STRIP IS INADEQUATE
? COULD BE ANY "RE" DILUTED TO NEAR 20 ppb (CAN BE RUN IN TANDEM)

SHORT DAY 3


INFLUENT STRIPS
DAILY QC STRIP
RANGE-FINDING STRIP(S)
MATRIX SPIKE
STD03
STD07
STD16
STD40
FB
FB 1:10
NC
QC 1:1000
RI 1:1000
RI 1:2000
RI 1:4000
NC
RI 1:•
MSI-1
MSI-2
RIMS-1 1:?
RIMS-2 1:
RUN FIRST
• ONLY RUN IF FIRST RANGE-FINDING STRIP IS INADEQUATE
? COULD BE ANY "RE" DILUTED TO 5-25ppb RANGE

GRAB INFLUENT SAMPLE
(Weekly Collection)

RANGE-FINDING STRIPS

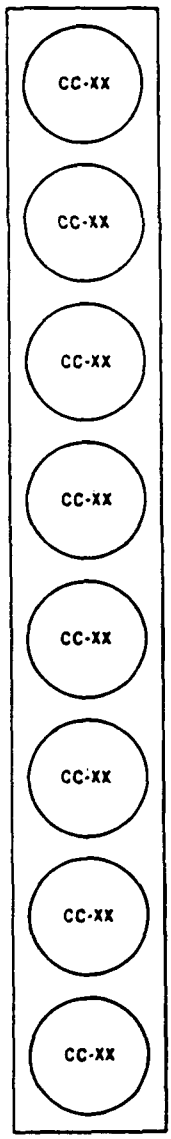


NOTE #1: THESE SAMPLES AREA TO BE ANALYZED ON DAY COLLECTED. IF POSSIBLE.

NOTE #2: IF NO OTHER DAILY QC STRIP IS RUN ON THE DAY THIS SAMPLE IS ANALYZED. INCLUDE A QC STRIP IN THE ANALYSIS.

NOTE #3: THIS ANALYSIS REQUIRES A SEPARATE FIELD FORM TO BE COMPLETED.

MICROWELL READER SPECTROPHOTOMETER
CROSS CALIBRATION



APPENDIX H

INSTRUCTIONS FOR HANDLING AND ANALYZING QA AND QC SAMPLES

INSTRUCTIONS FOR IMMUNOASSAY ANALYSIS OF QA AUDIT SAMPLE TYPES A AND B

1. Store all QA standard ampoules at 4 °C until use. Allow them to equilibrate at ambient temperature before opening. Break open a new ampoule for each analysis.

2. Break open an ampoule of Type A or B QA audit sample and, using a micropipettor, make 3 serial 10-fold dilutions as described in the Field Kit SOP, under influent sample dilution. The final dilution will be 1 mL of a 1 to 1000 dilution.

3. Label two more tubes with the sample number. Label one of the tubes with 1 to 2000 and one with 1 to 4000. Add 0.5 mL of 1X buffer to each of the tubes.

4. Pipet 0.5 mL from the 1 to 1000 tube into the 1 to 2000 tube and mix. Pipet 0.5 mL from the 1 to 2000 tube into the 1 to 4000 tube and mix.

5. Assay the 1 to 1000, 1 to 2000 and 1 to 4000 dilutions of the QA samples using the standard kit protocol on duplicate strips.

INSTRUCTIONS FOR HANDLING 20 PPM QC PERFORMANCE STANDARD

1. Store the sealed ampoules at 4 °C until use.

2. Allow the ampoule to equilibrate at room temperature before breaking open.

3. At the beginning of each week, break open a new ampoule (containing 2 mL) and transfer about 1 mL from the ampoule into two 1.8 mL glass auto-sampler vials. Label the vials with "20 ppm QC Performance Standard" and the date. Cap the vials with a Teflon-lined septum (red side down) and plastic screw cap. Store at 4 °C after each use.

4. Each day, pipet 100 uL out of the vial and make three log (dilute 0.1 mL to 1.0 mL) serial dilutions as described in the field kit analysis instructions.

5. Tightly recap the vial with Teflon (red) side facing the inside of the bottle and store at 4 °C until the next day's use.

INSTRUCTIONS FOR HANDLING THE 15 PPM STANDARD FOR MAKING INTERNAL STANDARD SPIKES

1. Store the sealed ampoules at 4 °C until use.

2. Allow the ampoule to equilibrate at room temperature before breaking open.

3. At the beginning of each week, break open a new ampoule (containing 1 mL) and transfer the contents into two 1.8 mL glass vials with a Teflon-lined (red side) cap and label with "15 ppm Standard for Spikes" and the date.

4. Each day, after allowing the contents to reach room temperature, pipet 100 uL of the standard into a tube containing 1.8 mL of 1X dilution buffer and mix. Label as "0.75 ppm Standard for Spiking." Proceed as described in the "Instructions for Field PCP Kit Analysis."

5. After use, tightly recap the vial with Teflon (red) side facing the inside of the bottle and store at 4 °C until the next use.

INSTRUCTIONS FOR RUNNING TYPE A AND B AUDIT SAMPLES IN THE LABORATORY PLATE FOR ELISA FOR PCP

1. Store ampules at 4 °C. allow them to equilibrate to room temperature before opening.

2. Break open a type A or B QA sample ampule and transfer the contents into a glass test tube.

3. Pipet 1.0 mL (volumetric) of the sample into a 10 mL volumetric flask and fill to the mark with 2-propanol, and mix. Transfer the remainder of the sample into a glass auto-sampler vial with a Teflon-lined screw cap, label and store at 4 °C.

4. Pipet 2.5 mL of the 10-fold dilution in 2-propanol made in step #3 into a 10 mL volumetric flask and bring to the mark with 25% 2-propanol in dilution buffer, and mix.

5. Pipet 2.5 mL of the dilution made in step #4, into a 5.0 mL volumetric and bring to the mark with 25% 2-propanol in dilution buffer.

6. Using the same procedure described in step #5, prepare two more serial 2-fold dilutions in 25% 2-propanol in dilution buffer.

7. Assay the dilutions made in step #4 and the three serial 2-fold dilutions made in steps #5 and #6 in triplicate wells in the PCP plate ELISA.

APPENDIX I

MATERIAL SAFETY DATA SHEETS (MSDS) FOR PENTACHLOROPHENOL AND METHANOL

MATERIAL SAFETY DATA SHEET OHS18150

OCCUPATIONAL HEALTH SERVICES, INC.
11 WEST 42ND STREET, 12TH FLOOR
NEW YORK, NEW YORK 10036
1-800-445-MSDS (1-800-445-6737) OR 1-212-789-35

FOR EMERGENCY SOURCE INFORMATION
CONTACT: 1-615-366-2000

SUBSTANCE IDENTIFICATION

CAS-NUMBER 87-86-5
RTEC-NUMBER SM6300000

SUBSTANCE: PENTACHLOROPHENOL

TRADE NAMES/SYNONYMS:
PHENOL, PENTACHLORO-: DOWICIDE 7: FUNGIFEN:
1-HYDROXYPENTACHLOROBENZENE: LAUXTOL: LIROPREM: PCP: PENCHLOROL:
PERMASAN: SANTOPHEN 20: STCC 4961380: RCRA U242: NA 2020:
C6HCL5O: OHS18150

CHEMICAL FAMILY:
HALOGEN COMPOUND, AROMATIC

MOLECULAR FORMULA: CL5-C6-O-H MOLECULAR WEIGHT: 266.34

CERCLA RATINGS (SCALE 0-3): HEALTH=3 FIRE=0 REACTIVITY=0 PERSISTENCE=3
NFPA RATINGS (SCALE 0-4): HEALTH=3 FIRE=0 REACTIVITY=0

COMPONENTS AND CONTAMINANTS

COMPONENT: PENTACHLOROPHENOL CAS# 87-86-5 PERCENT: 100.0

OTHER CONTAMINANTS: TECHNICAL GRADE MATERIAL MAY CONTAIN TRACES OF CHLORINATED DIBENZODIOXINS

EXPOSURE LIMIT:
PENTACHLOROPHENOL:
0.5 MG/M3 OSHA TWA (SKIN)
0.5 MG/M3 ACGIH TWA (SKIN)

10 POUNDS CERCLA SECTION 103 REPORTABLE QUANTITY
SUBJECT TO SARA SECTION 313 ANNUAL TOXIC CHEMICAL RELEASE REPORTING
SUBJECT TO CALIFORNIA PROPOSITION 65 CANCER AND/OR REPRODUCTIVE TOXICITY WARNING AND RELEASE REQUIREMENTS- (JANUARY 1, 1990)

PHYSICAL DATA

DESCRIPTION: WHITE POWDER OR CRYSTALS OR DARK-COLORED FLAKES WITH A VERY PUNGENT ODOR WHEN HOT.

BOILING POINT: 588-590 F (309-310 C)
@ 754 MMHG (DEC) MELTING POINT: 374-376 F (190-191 C)

SPECIFIC GRAVITY: 1.978 @ 22 C SOLUBILITY IN WATER: 14 PPM @ 20 C

VAPOR DENSITY: 9.2 VAPOR PRESSURE: 0.00017 MMHG @ 20 C

OTHER SOLVENTS (SOLVENT - SOLUBILITY):
SOLUBLE IN ALCOHOL, ETHER, BENZENE, CARBITOL, XYLENE, CELLOSOLVE, DIETHYLENE GLYCOL, PARAFFINIC PETROLEUM OILS, DILUTE ALKALI; MODERATELY SOLUBLE IN ETHYLENE GLYCOL, CARBON TETRACHLORIDE.

OTHER PHYSICAL DATA
TECHNICAL GRADE MATERIAL MAY MELT ABOVE 338 F (170 C)

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD
NEGLIGIBLE FIRE HAZARD WHEN EXPOSED TO HEAT OR FLAME.

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR REGULAR FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR REGULAR FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5, GUIDE PAGE 53).

USE AGENTS SUITABLE FOR TYPE OF FIRE. AVOID BREATHING HAZARDOUS VAPORS, KEEP UPWIND.

TRANSPORTATION

DEPARTMENT OF TRANSPORTATION HAZARD CLASSIFICATION 49 CFR 172.101:
POISON B

DEPARTMENT OF TRANSPORTATION LABELING REQUIREMENTS 49 CFR 172.101 AND SUBPART E:
POISON

DEPARTMENT OF TRANSPORTATION PACKAGING REQUIREMENTS: 49 CFR 173.365
EXCEPTIONS: 49 CFR 173.364

TOXICITY

PENTACHLOROPHENOL:

IRRITATION DATA: 10 MG/24 HOURS OPEN SKIN-RABBIT MILD.

TOXICITY DATA: 355 MG/M3 INHALATION-RAT LC50; 225 MG/M3 INHALATION-MOUSE LC50; 40 MG/KG SKIN-RABBIT LDLO; 96 MG/KG SKIN-RAT LD50; 401 MG/KG ORAL-MAN LDLO; 27 MG/KG ORAL-RAT LD50; 117 MG/KG ORAL-MOUSE LD50; 70 MG/KG ORAL-RABBIT LDLO; 168 MG/KG ORAL-HAMSTER LD50; 100 MG/KG SUBCUTANEOUS-RAT LD50; 70 MG/KG SUBCUTANEOUS-RABBIT LDLO; 135 MG/KG SUBCUTANEOUS-DOG LDLO; 56 MG/KG INTRAPERITONEAL-RAT LD50; 58 MG/KG INTRAPERITONEAL-MOUSE LD50; 135 MG/KG INTRAPERITONEAL-RABBIT LDLO; 100 MG/KG UNREPORTED-GUINEA PIG LD50; 70 MG/KG UNREPORTED-DOG LD50; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS); TUMORIGENIC DATA (RTECS).

CARCINOGEN STATUS: HUMAN LIMITED EVIDENCE (IARC GROUP-2B FOR CHLOROPHENOLS); ANIMAL INADEQUATE EVIDENCE (IARC). STUDIES REVEALED A SIGNIFICANT INCREASE IN SOFT-TISSUE SARCOMA AND LUNG, NASAL AND NASOPHARYNGEAL CANCER IN WORKERS EXPOSED TO CHLOROPHENOLS. THERE WAS CLEAR EVIDENCE OF CARCINOGENIC ACTIVITY IN MICE FED A TECHNICAL-GRADE AND A TECHNICAL-GRADE FORMULATION AS SHOWN BY INCREASED INCIDENCES OF ADRENAL MEDULLARY AND HEPATOCELLULAR NEOPLASMS AND HEMANGIOSARCOMAS (NTP TR-349).

LOCAL EFFECTS: IRRITANT- INHALATION, SKIN, EYE.

ACUTE TOXICITY LEVEL: HIGHLY TOXIC BY INHALATION, DERMAL ABSORPTION AND INGESTION.

TARGET EFFECTS: POISONING MAY INCREASE THE METABOLIC RATE AND AFFECT THE CARDIOVASCULAR AND NERVOUS SYSTEMS, LIVER, AND KIDNEYS.

AT INCREASED RISK FROM EXPOSURE: PERSONS WITH RENAL OR HEPATIC DISEASES.

ADDITIONAL DATA: HOT ENVIRONMENTS MAY ENHANCE ABSORPTION AND THE TOXIC EFFECTS.

HEALTH EFFECTS AND FIRST AID

INHALATION:
PENTACHLOROPHENOL:
IRRITANT/HIGHLY TOXIC.
150 MG/M3 IMMEDIATELY DANGEROUS TO LIFE OR HEALTH.
ACUTE EXPOSURE- MAY CAUSE IRRITATION OF THE UPPER RESPIRATORY TRACT WITH CONCENTRATIONS GREATER THAN 1 MG/M3 PRODUCING PAIN IN THE NOSE AND THROAT, VIOLENT SNEEZING, AND COUGH. SYMPTOMS OF SYSTEMIC POISONING MAY INCLUDE HEADACHE, FEVER, INTENSE THIRST, EXCESSIVE PERSPIRATION, GENERALIZED WEAKNESS, DIZZINESS, TACHYCARDIA, TACHYPNEA, DYSPNEA, CHEST PAIN, PAIN IN THE EXTREMITIES, ANOREXIA, WEIGHT LOSS, METABOLIC ACIDOSIS, AND GASTROINTESTINAL UPSET WITH NAUSEA, VOMITING, AND ABDOMINAL PAIN. IN SEVERE POISONINGS, THESE EFFECTS MAY PROGRESS TO MUSCLE SPASM, DEHYDRATION, HYPERPYREXIA, ANESTHESIA, LEUKOCYTOSIS, HYPERGLYCEMIA, EDEMA AND HEMORRHAGE IN THE LUNGS, CEREBRAL EDEMA, STUPOR, CONVULSIONS, AND COMA. LIVER AND KIDNEY DAMAGE MAY OCCUR. DEATH MAY BE DUE TO VASCULAR COLLAPSE AND HEART FAILURE AND MAY OCCUR WITHIN HOURS OF THE ONSET OF SYMPTOMS FOLLOWED RAPIDLY BY RIGOR MORTIS. IMPAIRMENT OF AUTONOMIC FUNCTION AND CIRCULATION AND VISUAL DAMAGE WERE OBSERVED IN SOME SERIOUS CASES OF POISONING.
CHRONIC EXPOSURE- REPEATED EXPOSURE TO LOW-LEVELS MAY CAUSE IRRITATION OF THE NOSE, THROAT, AND LUNGS LEADING TO BRONCHITIS AND SINUSITIS. IN ADDITION TO THE SYSTEMIC EFFECTS LISTED ABOVE, REPEATED OR PROLONGED EXPOSURE HAS BEEN ASSOCIATED WITH THE DEVELOPMENT OF ACUTE PANCREATITIS, LEUKOPENIA, IMMUNOLOGICAL CHANGES, APLASTIC ANEMIA, INTRAVASCULAR HEMOLYSIS, AND POLYNEURITIS.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION. ADMINISTER OXYGEN. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
PENTACHLOROPHENOL:
IRRITANT/HIGHLY TOXIC.
ACUTE EXPOSURE- BRIEF, SINGLE EXPOSURE TO SOLUTIONS CONTAINING APPROXIMATELY 10% PENTACHLOROPHENOL MAY CAUSE IRRITATION. SOLIDS AND CONCENTRATED SOLUTIONS MAY POSSIBLY PRODUCE SKIN BURNS. THIS MATERIAL MAY BE ABSORBED THROUGH THE SKIN IN FATAL AMOUNTS AND PRODUCE SYSTEMIC EFFECTS AS DESCRIBED IN ACUTE INHALATION.

CHRONIC EXPOSURE- PROLONGED OR REPEATED EXPOSURE MAY CAUSE DERMATITIS AND A RARE ALLERGIC SKIN RESPONSE; SOLUTIONS CONTAINING AS LITTLE AS 1% MAY CAUSE IRRITATION. REPEATED ABSORPTION MAY RESULT IN SYSTEMIC EFFECTS AS DESCRIBED IN INHALATION. CHLORACNE AND DISORDERS OF THE NERVOUS SYSTEM, LIVER, AND PORPHYRIA MAY OCCUR DUE TO THE PRESENCE OF CHLORINATED DIBENZODIOXINS.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. THEN REMOVE SKIN AND HAIR CONTAMINATION BY SCRUBBING WITH SOAP AND WATER. IF BODY TEMPERATURE IS ELEVATED, REDUCE TO 37 C BY SPONGE BATH, IMMERSION IN COOL WATER OR BY APPLYING COOLING BLANKET. IF BODY TEMPERATURE IS ABOVE 40 C, ICE WATER IS NECESSARY (DREISBACH, HANDBOOK OF POISONING, 12TH EDITION; MORGAN, EPA RECOGNITION AND MANAGEMENT OF PESTICIDE POISONINGS, 3RD EDITION). GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
PENTACHLOROPHENOL:
IRRITANT.
ACUTE EXPOSURE- EXPOSURE TO FINE DUST AND SPRAYS MAY CAUSE PAINFUL IRRITATION, LACRIMATION, CORNEAL NUMBNESS, SLIGHT MYDRIASIS, AND INFLAMMATION THAT MAY PROGRESS TO PERMANENT CORNEAL INJURY.
CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE MAY CAUSE CONJUNCTIVITIS.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER OR NORMAL SALINE, OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:
PENTACHLOROPHENOL:
HIGHLY TOXIC.
ACUTE EXPOSURE- MAY CAUSE SEVERE IRRITATION OF THE GASTROINTESTINAL TRACT AND SYSTEMIC EFFECTS AS DESCRIBED IN INHALATION. SEVERE TOXIC EFFECTS MAY OCCUR IN HUMANS WITH INGESTION OF 2 GRAMS.
CHRONIC EXPOSURE- MAY CAUSE EFFECTS AS DESCRIBED IN INHALATION. ENLARGEMENT OF THE LIVER, CHANGES IN VARIOUS ENZYME ACTIVITIES, AND OTHER HEPATIC EFFECTS WERE OBSERVED IN RATS RECEIVING PENTACHLOROPHENOL FOR PERIODS OF THREE TO EIGHT MONTHS. FETOTOXIC EFFECTS, FETAL DEATHS AND RESORPTIONS HAVE BEEN REPORTED IN RODENTS. INCREASED INCIDENCES OF ADRENAL MEDULLARY AND HEPATOCELLULAR NEOPLASMS AND HEMANGIOSARCOMAS WERE OBSERVED IN A 2-YEAR STUDY OF MICE.

FIRST AID- IF VICTIM IS ALERT AND RESPIRATION IS NOT DEPRESSED, INDUCE EMESIS WITH SYRUP OF IPECAC. IF VICTIM IS NOT FULLY ALERT, EMPTY THE STOMACH IMMEDIATELY BY INTUBATION, ASPIRATION, AND LAVAGE, USING ISOTONIC

SALINE OR 5% SODIUM BICARBONATE. FOLLOW EMESIS OR LAVAGE WITH ACTIVATED CHARCOAL. GIVE SODIUM SULFATE AS A CATHARTIC. REDUCE ELEVATED BODY TEMPERATURE TO 37 C BY SPONGE BATHS, IMMERSION IN COOL WATER OR BY APPLYING COOLING BLANKET. (MORGAN, EPA RECOGNITION AND MANAGEMENT OF PESTICIDE POISONINGS, THIRD EDITION). GET MEDICAL ATTENTION.

ANTIDOTE:
NO SPECIFIC ANTIDOTE. TREAT SYMPTOMATICALLY AND SUPPORTIVELY.

REACTIVITY SECTION

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES.

INCOMPATIBILITIES:
PENTACHLOROPHENOL:
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.

DECOMPOSITION:
THERMAL DECOMPOSITION PRODUCTS MAY INCLUDE TOXIC AND CORROSIVE CHLORIDE FUMES, TOXIC AND HAZARDOUS CHLORINATED PHENOLS AND OXIDES OF CARBON.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE-DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

STORAGE

PROTECT AGAINST PHYSICAL DAMAGE. STORE IN A COOL, DRY, WELL VENTILATED LOCATION, AWAY FROM ANY AREA WHERE THE FIRE HAZARD MAY BE ACUTE. OUTSIDE OR DETACHED STORAGE IS PREFERRED (NFPA 49, HAZARDOUS CHEMICALS DATA, 1975).

STORE AT 4 C, PROTECT FROM LIGHT AND KEEP DRY.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

DISPOSAL

PENTACHLOROPHENOL - REGULATORY LEVEL: 100.0 MG/L
MATERIALS WHICH CONTAIN THE ABOVE SUBSTANCE AT OR ABOVE THE REGULATORY LEVEL MEET THE EPA CHARACTERISTIC OF TOXICITY, AND MUST BE DISPOSED OF IN ACCORDANCE WITH 40 CFR PART 262. EPA HAZARDOUS WASTE NUMBER D037.

CONDITIONS TO AVOID

MAY BURN BUT DOES NOT IGNITE READILY. PREVENT DISPERSION OF DUST IN AIR. DO NOT ALLOW SPILLED MATERIAL TO CONTAMINATE WATER SOURCES.

SPILLS AND LEAKS

SOIL-RELEASE:
DIG A PIT, POND, LAGOON OR HOLDING AREA TO CONTAIN LIQUID OR SOLID MATERIAL. COVER SOLIDS WITH A PLASTIC SHEET TO PREVENT DISSOLVING IN RAIN OR FIREFIGHTING WATER.

WATER-SPILL:
USE NATURAL DEEP WATER POCKETS, EXCAVATED LAGOONS, OR SAND BAG BARRIERS TO TRAP MATERIAL AT BOTTOM. USE ACTIVATED CARBON AT 10 TIMES THE SPILLED AMOUNT IF IT IS DISSOLVED AT 10 PPM OR GREATER CONCENTRATION. REMOVE TRAPPED MATERIAL WITH SUCTION HOSES. USE MECHANICAL DREDGES OR LIFTS TO REMOVE IMMOBILIZED MASSES OF POLLUTION AND PRECIPITATES.

THE CALIFORNIA SAFE DRINKING WATER AND TOXIC ENFORCEMENT ACT OF 1986 (PROPOSITION 65) PROHIBITS CONTAMINATING ANY KNOWN SOURCE OF DRINKING WATER WITH SUBSTANCES KNOWN TO CAUSE CANCER AND/OR REPRODUCTIVE TOXICITY.

OCCUPATIONAL-SPILL:
DO NOT TOUCH SPILLED MATERIAL. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR SMALL DRY SPILLS, WITH A CLEAN SHOVEL PLACE MATERIAL INTO CLEAN, DRY CONTAINER AND COVER. MOVE CONTAINERS FROM SPILL AREA. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. KEEP UNNECESSARY PEOPLE AWAY. ISOLATE HAZARD AREA AND DENY ENTRY.

REPORTABLE QUANTITY (RQ): 10 POUNDS
THE SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT (SARA) SECTION 304 REQUIRES THAT A RELEASE EQUAL TO OR GREATER THAN THE REPORTABLE QUANTITY FOR THIS SUBSTANCE BE IMMEDIATELY REPORTED TO THE LOCAL EMERGENCY PLANNING COMMITTEE AND THE STATE EMERGENCY RESPONSE COMMISSION (40 CFR 355.40). IF THE RELEASE OF

THIS SUBSTANCE IS REPORTABLE UNDER CERCLA SECTION 103, THE NATIONAL RESPONSE CENTER MUST BE NOTIFIED IMMEDIATELY AT (800) 424-8802 OR (202) 426-2675 IN THE METROPOLITAN WASHINGTON, D.C. AREA (40 CFR 302.6).

PROTECTIVE EQUIPMENT SECTION

VENTILATION:
PROCESS ENCLOSURE RECOMMENDED TO MEET PUBLISHED EXPOSURE LIMITS.

RESPIRATOR:
THE FOLLOWING RESPIRATORS AND MAXIMUM USE CONCENTRATIONS ARE RECOMMENDATIONS BY THE U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, NIOSH POCKET GUIDE TO CHEMICAL HAZARDS; NIOSH CRITERIA DOCUMENTS OR BY THE U.S. DEPARTMENT OF LABOR, 29 CFR 1910 SUBPART Z.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

PENTACHLOROPHENOL:

5.0 MG/M3- CHEMICAL CARTRIDGE RESPIRATOR WITH ORGANIC VAPOR CARTRIDGE(S) IN COMBINATION WITH A DUST, MIST, AND FUME FILTER.
SUPPLIED-AIR RESPIRATOR.
SELF-CONTAINED BREATHING APPARATUS.

12.5 MG/M3- SUPPLIED-AIR RESPIRATOR OPERATED IN CONTINUOUS FLOW MODE.
POWERED AIR-PURIFYING RESPIRATOR WITH ORGANIC VAPOR CARTRIDGE(S) IN COMBINATION WITH A DUST, MIST, AND FUME FILTER.

25.0 MG/M3- CHEMICAL CARTRIDGE RESPIRATOR WITH FULL FACEPIECE AND ORGANIC VAPOR CARTRIDGE(S) IN COMBINATION WITH A HIGH-EFFICIENCY PARTICULATE FILTER.
SUPPLIED-AIR RESPIRATOR WITH FULL FACEPIECE.
SELF-CONTAINED BREATHING APPARATUS WITH FULL FACEPIECE.

150 MG/M3- SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE AND OPERATED IN PRESSURE DEMAND OR OTHER POSITIVE PRESSURE MODE.

ESCAPE- AIR-PURIFYING FULL FACEPIECE RESPIRATOR (GAS MASK) WITH A CHIN-STYLE OR FRONT- OR BACK-MOUNTED ORGANIC VAPOR CANISTER HAVING A HIGH-EFFICIENCY PARTICULATE FILTER.
ESCAPE-TYPE SELF-CONTAINED BREATHING APPARATUS.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

SELF-CONTAINED BREATHING APPARATUS WITH FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

SUPPLIED-AIR RESPIRATOR WITH FULL FACEPIECE AND OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT ANY POSSIBILITY OF SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES AND A FACESHIELD TO PREVENT CONTACT WITH THIS SUBSTANCE.

EMERGENCY WASH FACILITIES:
WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES AND/OR SKIN MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN AND QUICK DRENCH SHOWER WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

AUTHORIZED BY- OCCUPATIONAL HEALTH SERVICES, INC.

CREATION DATE: 03/18/85 REVISION DATE: 10/10/90

MATERIAL SAFETY DATA SHEET OHS14280

OCCUPATIONAL HEALTH SERVICES, INC.
11 WEST 42ND STREET, 12TH FLOOR
NEW YORK, NEW YORK 10036
1-800-445-MSDS (1-800-445-6737) OR 1-212-789-3535

FOR EMERGENCY SOURCE INFORMATION
CONTACT: 1-615-366-2000

SUBSTANCE IDENTIFICATION

CAS-NUMBER 67-56-1
RTEC-NUMBER PC1400000

SUBSTANCE: METHYL ALCOHOL

TRADE NAMES/SYNONYMS:
METHANOL: WOOD ALCOHOL: METHYL HYDROXIDE: CARBINOL: MONOHYDROXYMETHANE: WOOD SPIRIT: WOOD NAPHTHA: METHYLOL: COLONIAL SPIRIT: COLUMBIAN SPIRIT: PYROXYLIC SPIRIT: BOOSTER FUEL (HENES PRODUCT CORP.): METHANOL (ELECTROKLEIN) (ROK): METHANOL, SPECTRO QUALITY (MCB MANF. CHEMIST): COULOMATIC (R) CONDITIONER SOLUTION: STANDARD WATER IN METHANOL: STCC 4904230: RCRA U154: UN 1230: CH4O: OHS14280

CHEMICAL FAMILY:
HYDROXYL, ALIPHATIC

MOLECULAR FORMULA: C-H3-O-H MOLECULAR WEIGHT: 32.04

CERCLA RATINGS (SCALE 0-3): HEALTH=3 FIRE=3 REACTIVITY=0 PERSISTENCE=0
NFPA RATINGS (SCALE 0-4): HEALTH=1 FIRE=3 REACTIVITY=0

COMPONENTS AND CONTAMINANTS

COMPONENT: METHYL ALCOHOL (METHANOL) CAS# 67-56-1 PERCENT: 100

OTHER CONTAMINANTS: NONE

EXPOSURE LIMIT:
METHYL ALCOHOL (METHANOL):
200 PPM (260 MG/M3) OSHA TWA (SKIN); 250 PPM (325 MG/M3) OSHA STEL
200 PPM (260 MG/M3) ACGIH TWA (SKIN); 250 PPM (310 MG/M3) ACGIH STEL
200 PPM NIOSH RECOMMENDED 10 HOUR TWA;
800 PPM NIOSH RECOMMENDED 15 MINUTE CEILING

5000 POUNDS CERCLA SECTION 103 REPORTABLE QUANTITY
SUBJECT TO SARA SECTION 313 ANNUAL TOXIC CHEMICAL RELEASE REPORTING

PHYSICAL DATA

DESCRIPTION: CLEAR, COLORLESS LIQUID WITH A CHARACTERISTIC ALCOHOLIC ODOR.

BOILING POINT: 149 F (65 C) MELTING POINT: -137 F (-94 C)

SPECIFIC GRAVITY: 0.7914 EVAPORATION RATE: 4.6 (BUTYL ACETATE=1)

VISCOSITY: 0.59 CPS @ 20 C SOLUBILITY IN WATER: VERY SOLUBLE

VAPOR DENSITY: 1.11 VAPOR PRESSURE: 97.25 MMHG @ 20 C

ODOR-THRESHOLD: 100 PPM

OTHER SOLVENTS (SOLVENT - SOLUBILITY):
SOLUBLE IN ETHER, BENZENE, ALCOHOL, ACETONE,
CHLOROFORM, ETHANOL, KETONES AND MOST OTHER ORGANIC SOLVENTS.

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD
DANGEROUS FIRE HAZARD WHEN EXPOSED TO HEAT, FLAME, OR OXIDIZERS.

VAPORS ARE HEAVIER THAN AIR AND MAY TRAVEL A CONSIDERABLE DISTANCE TO A SOURCE OF IGNITION AND FLASH BACK.

VAPOR-AIR MIXTURES ARE EXPLOSIVE.

FLASH POINT: 52 F (11 C) (CC) UPPER EXPLOSION LIMIT: 36.0%

LOWER EXPLOSION LIMIT: 6.0% AUTOIGNITION TEMP.: 725 F (385 C)

FLAMMABILITY CLASS (OSHA): IB

FIREFIGHTING MEDIA:
DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FOR LARGER FIRES, USE WATER SPRAY, FOG OR ALCOHOL-RESISTANT FOAM (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5).

FIREFIGHTING:
MOVE CONTAINER FROM FIRE AREA IF YOU CAN DO IT WITHOUT RISK. DIKE FIRE-CONTROL WATER FOR LATER DISPOSAL; DO NOT SCATTER THE MATERIAL. APPLY COOLING WATER TO SIDES OF CONTAINERS THAT ARE EXPOSED TO FLAMES UNTIL WELL AFTER FIRE IS OUT. STAY AWAY FROM ENDS OF TANKS. WITHDRAW IMMEDIATELY IN CASE OF RISING SOUND

FROM VENTING SAFETY DEVICE OR ANY DISCOLORATION OF TANK DUE TO FIRE. ISOLATE FOR 1/2 MILE IN ALL DIRECTIONS IF TANK, RAIL CAR OR TANK TRUCK IS INVOLVED IN FIRE (1990 EMERGENCY RESPONSE GUIDEBOOK, DOT P 5800.5, GUIDE PAGE 28).

EXTINGUISH ONLY IF FLOW CAN BE STOPPED; USE WATER IN FLOODING AMOUNTS AS FOG, SOLID STREAMS MAY NOT BE EFFECTIVE. COOL CONTAINERS WITH FLOODING QUANTITIES OF WATER, APPLY FROM AS FAR A DISTANCE AS POSSIBLE. AVOID BREATHING TOXIC VAPORS, KEEP UPWIND.

TRANSPORTATION

DEPARTMENT OF TRANSPORTATION HAZARD CLASSIFICATION 49 CFR 172.101:
FLAMMABLE LIQUID

DEPARTMENT OF TRANSPORTATION LABELING REQUIREMENTS 49 CFR 172.101 AND SUBPART E:
FLAMMABLE LIQUID

DEPARTMENT OF TRANSPORTATION PACKAGING REQUIREMENTS: 49 CFR 173.119
EXCEPTIONS: 49 CFR 173.118

TOXICITY

METHYL ALCOHOL (METHANOL):

IRRITATION DATA: 20 MG/24 HOURS SKIN-RABBIT MODERATE; 40 MG EYE-RABBIT MODERATE; 100 MG/24 HOURS EYE-RABBIT MODERATE.

TOXICITY DATA: 86,000 MG/M3 INHALATION-HUMAN TCLO; 300 PPM INHALATION-HUMAN TCLO; 64,000 PPM/4 HOURS INHALATION-RAT LC50; 1000 PPM INHALATION-MONKEY LCLO; 50 GM/M3/2 HOURS INHALATION-MOUSE LCLO; 44,000 MG/M3/6 HOURS INHALATION-CAT LCLO; 15,800 MG/KG SKIN-RABBIT LD50; 393 MG/KG SKIN-MONKEY LDLO; 428 MG/KG ORAL-HUMAN LDLO; 143 MG/KG ORAL-HUMAN LDLO; 6422 MG/KG ORAL-MAN LDLO; 3429 MG/KG ORAL-MAN TDLO; 4 GM/KG ORAL-WOMAN TDLO; 7 GM/KG ORAL-MONKEY LD50; 5628 MG/KG ORAL-RAT LD50; 7300 MG/KG ORAL-MOUSE LD50; 14,200 MG/KG ORAL-RABBIT LD50; 7500 MG/KG ORAL-DOG LDLO; 9800 MG/KG SUBCUTANEOUS-MOUSE LD50; 2131 MG/KG INTRAVENOUS-RAT LD50; 4710 MG/KG INTRAVENOUS-MOUSE LD50; 8907 MG/KG INTRAVENOUS-RABBIT LD50; 7529 MG/KG INTRAPERITONEAL-RAT LD50; 10,765 MG/KG INTRAPERITONEAL-MOUSE LD50; 1826 MG/KG INTRAPERITONEAL-RABBIT LD50; 868 MG/KG UNREPORTED-MAN LDLO; MUTAGENIC DATA (RTECS); REPRODUCTIVE EFFECTS DATA (RTECS).
CARCINOGEN STATUS: NONE.
LOCAL EFFECTS: IRRITANT- SKIN, EYE.

ACUTE TOXICITY LEVEL: SLIGHTLY TOXIC BY INHALATION, DERMAL ABSORPTION, INGESTION.

TARGET EFFECTS: NEUROTOXIN; CENTRAL NERVOUS SYSTEM DEPRESSANT.
AT INCREASED RISK FROM EXPOSURE: PERSONS WITH KIDNEY, EYE OR SKIN DISORDERS.

HEALTH EFFECTS AND FIRST AID

INHALATION:
METHYL ALCOHOL (METHANOL):
NARCOTIC/NEUROTOXIN. 25,000 PPM IMMEDIATELY DANGEROUS TO LIFE OR HEALTH.
ACUTE EXPOSURE- MAY CAUSE IRRITATION OF THE MUCOUS MEMBRANES, COUGHING, OPPRESSION IN THE CHEST, TRACHEITIS, BRONCHITIS, TINNITUS, UNSTEADY GAIT, TWITCHING, COLIC, CONSTIPATION, NYSTAGMUS, AND BLEPHAROSPASM. SYMPTOMS FROM OCCUPATIONAL EXPOSURE INCLUDE PARESTHESIAS, NUMBNESS AND SHOOTING PAINS IN THE HANDS AND FOREARMS. METABOLIC ACIDOSIS, AND EFFECTS ON THE EYES AND CENTRAL NERVOUS SYSTEM MAY OCCUR AS DETAILED IN ACUTE INGESTION.
CHRONIC EXPOSURE- REPEATED OR PROLONGED EXPOSURE MAY CAUSE EFFECTS AS IN ACUTE INGESTION. REPEATED EXPOSURE TO 200-375 PPM CAUSED RECURRENT HEADACHES IN WORKERS. EXPOSURE FOR 4 YEARS TO 1200-8000 PPM RESULTED IN MARKED DIMINUTION OF VISION AND ENLARGEMENT OF THE LIVER IN A WORKMAN. REPRODUCTIVE EFFECTS HAVE BEEN REPORTED IN ANIMALS.

FIRST AID- REMOVE FROM EXPOSURE AREA TO FRESH AIR IMMEDIATELY. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION. KEEP PERSON WARM AND AT REST. TREAT SYMPTOMATICALLY AND SUPPORTIVELY. GET MEDICAL ATTENTION IMMEDIATELY.

SKIN CONTACT:
METHYL ALCOHOL (METHANOL):
IRRITANT/NARCOTIC/NEUROTOXIN.
ACUTE EXPOSURE- CONTACT WITH LIQUID MAY CAUSE IRRITATION. SKIN ABSORPTION MAY OCCUR AND CAUSE METABOLIC ACIDOSIS AND EFFECTS ON THE EYES AND CENTRAL NERVOUS SYSTEM AS DETAILED IN ACUTE INGESTION.
CHRONIC EXPOSURE- REPEATED OR PROLONGED CONTACT WITH THE LIQUID MAY CAUSE DEFATTING OF THE SKIN RESULTING IN ERYTHEMA, SCALING, AND ECZEMATOID DERMATITIS. CHRONIC ABSORPTION MAY RESULT METABOLIC ACIDOSIS AND EFFECTS AS DETAILED IN ACUTE INGESTION.

FIRST AID- REMOVE CONTAMINATED CLOTHING AND SHOES IMMEDIATELY. WASH AFFECTED AREA WITH SOAP OR MILD DETERGENT AND LARGE AMOUNTS OF WATER UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

EYE CONTACT:
METHYL ALCOHOL (METHANOL):
IRRITANT.
ACUTE EXPOSURE- VAPORS MAY CAUSE IRRITATION. HIGH CONCENTRATIONS HAVE BEEN REPORTED TO CAUSE VIOLENT INFLAMMATION OF THE CONJUNCTIVA AND

EPITHELIAL DEFECTS ON THE CORNEA. MILD IRRITATION MAY OCCUR WITH DILUTE SOLUTIONS; THE UNDILUTED LIQUID HAS PRODUCED MODERATE CORNEAL OPACITY AND CONJUNCTIVAL REDNESS IN RABBITS. APPLICATION OF A DROP OF METHANOL IN RABBIT EYES CAUSED A MILD REVERSIBLE REACTION, GRADED 3 ON A SCALE OF 1-10 AFTER 24 HOURS.

CHRONIC EXPOSURE- REPEATED OR PROLONGED CONTACT MAY CAUSE CONJUNCTIVITIS.

FIRST AID- WASH EYES IMMEDIATELY WITH LARGE AMOUNTS OF WATER OR NORMAL SALINE,

OCCASIONALLY LIFTING UPPER AND LOWER LIDS, UNTIL NO EVIDENCE OF CHEMICAL REMAINS (APPROXIMATELY 15-20 MINUTES). GET MEDICAL ATTENTION IMMEDIATELY.

INGESTION:

METHYL ALCOHOL (METHANOL):

NARCOTIC/NEUROTOXIN.

ACUTE EXPOSURE- MAY CAUSE MILD AND TRANSIENT INEBRIATION AND SUBSEQUENT DROWSINESS FOLLOWED BY AN ASYMPTOMATIC PERIOD LASTING 8-48 HOURS. FOLLOWING THE DELAY, COUGHING, DYSPNEA, HEADACHE, DULLNESS, WEAKNESS, VERTIGO OR DIZZINESS, NAUSEA, VOMITING, OCCASIONAL DIARRHEA, ANOREXIA, VIOLENT PAIN IN THE BACK, ABDOMEN, AND EXTREMITIES, RESTLESSNESS, APATHY OR DELIUIUM, AND RARELY, EXCITEMENT AND MANIA MAY OCCUR. RAPID, SHALLOW RESPIRATION DUE TO METABOLIC ACIDOSIS, COLD AND CLAMMY SKIN, HYPOTENSION, CYANOSIS, OPISTHOTONOS, CONVULSIONS, MILD TACHYCARDIA, CARDIAC DEPRESSION, PERIPHERAL NEURITIS, CEREBRAL AND PULMONARY EDEMA, UNCONSCIOUSNESS, AND COMA ARE POSSIBLE. EFFECTS ON THE EYE MAY INCLUDE OPTIC NEURITIS, BLURRED OR DIMMED VISION, DILATED, UNRESPONSIVE PUPILS, PTOSIS, EYE PAIN, CONCENTRIC CONSTRICTION OF VISUAL FIELDS, DIPLOPIA, CHANGE IN COLOR PERCEPTION, PHOTOPHOBIA, AND OPTIC NERVE ATROPHY. PARTIAL BLINDNESS OR POSSIBLY DELAYED TRANSIENT OR PERMANENT BLINDNESS MAY OCCUR. BILATERAL SENSORINEURAL DEAFNESS HAS BEEN REPORTED IN A SINGLE CASE. LIVER, KIDNEY, HEART, STOMACH, INTESTINAL AND PANCREATIC DAMAGE MAY ALSO OCCUR. DEATH MAY BE DUE TO RESPIRATORY FAILURE OR RARELY FROM CIRCULATORY COLLAPSE. AS LITTLE AS 15 ML HAS CAUSED BLINDNESS; THE USUAL FATAL DOSE IS 60-240 ML. PROLONGED ASTHENIA AND IRREVERSIBLE EFFECTS ON THE NERVOUS SYSTEM INCLUDING DIFFICULTY IN SPEECH, MOTOR DYSFUNCTION WITH RIGIDITY, SPASTICITY, AND HYPOKINESIS HAVE BEEN REPORTED.

CHRONIC EXPOSURE- REPEATED INGESTION MAY CAUSE VISUAL IMPAIRMENT AND BLINDNESS AND OTHER SYSTEMIC EFFECTS AS DETAILED IN ACUTE INGESTION. REPRODUCTIVE EFFECTS HAVE BEEN REPORTED IN ANIMALS.

FIRST AID- IF INGESTION OF METHANOL IS DISCOVERED WITHIN 2 HOURS, GIVE SYRUP OF IPECAC. LAVAGE THOROUGHLY WITH 2-4 L OF TAP WATER WITH SODIUM BICARBONATE (20 G/L) ADDED. GET MEDICAL ATTENTION IMMEDIATELY. LAVAGE SHOULD BE PERFORMED BY QUALIFIED MEDICAL PERSONNEL (DREISBACH, HANDBOOK OF POISONING, 12TH ED.).

ANTIDOTE:
THE FOLLOWING ANTIDOTE(S) HAVE BEEN RECOMMENDED. HOWEVER, THE DECISION AS TO WHETHER THE SEVERITY OF POISONING REQUIRES ADMINISTRATION OF ANY ANTIDOTE AND ACTUAL DOSE REQUIRED SHOULD BE MADE BY QUALIFIED MEDICAL PERSONNEL.

METHANOL POISONING:
GIVE ETHANOL, 50% (100 PROOF), 1.5 ML/KG ORALLY INITIALLY, DILUTED TO NOT MORE THAN 5% SOLUTION, FOLLOWED BY 0.5-1.0 ML/KG EVERY 2 HOURS ORALLY OR INTRAVENOUSLY FOR 4 DAYS IN ORDER TO REDUCE METABOLISM OF METHANOL AND TO ALLOW TIME FOR ITS EXCRETION. BLOOD ETHANOL LEVEL SHOULD BE IN THE RANGE OF 1-1.5 MG/ML (DREISBACH, HANDBOOK OF POISONING, 12TH ED.). ANTIDOTE SHOULD BE ADMINISTERED BY QUALIFIED MEDICAL PERSONNEL.

ORAL OR INTRAVENOUS ADMINISTRATION OF 4-METHYLPYRAZOLE INHIBITS ALCOHOL DEHYDROGENASE AND HAS BEEN USED EFFECTIVELY AS AN ANTIDOTE FOR METHANOL OR ETHYLENE GLYCOL POISONING (ELLENHORN AND BARCELOUX, MEDICAL TOXICOLOGY).

REACTIVITY SECTION

REACTIVITY:
STABLE UNDER NORMAL TEMPERATURES AND PRESSURES.

INCOMPATIBILITIES:
METHYL ALCOHOL (METHANOL):
ACETYL BROMIDE: VIOLENT REACTION WITH FORMATION OF HYDROGEN BROMIDE.
ALKYLALUMINUM SOLUTIONS: VIOLENT REACTION.
ALUMINUM: CORRODES.
BARIUM PERCHLORATE: DISTILLATION YIELDS HIGHLY EXPLOSIVE ALKYL PERCHLORATE.
BERYLLIUM HYDRIDE: VIOLENT REACTION, EVEN AT -196 C.
BROMINE: VIGOROUSLY EXOTHERMIC REACTION.
CALCIUM CARBIDE: VIOLENT REACTION.
CHLORINE: POSSIBLE IGNITION AND EXPLOSION HAZARD.
CHLOROFORM AND SODIUM HYDROXIDE: EXPLOSIVE REACTION.
CHROMIUM TRIOXIDE (CHROMIC ANHYDRIDE): POSSIBLE IGNITION.
CYANURIC CHLORIDE: VIOLENT REACTION.
DICHLOROMETHANE: POSSIBLE IGNITION AND EXPLOSION.
DIETHYL ZINC: POSSIBLE IGNITION AND EXPLOSION.
HYDROGEN PEROXIDE + WATER: EXPLOSION HAZARD.
IODINE + ETHANOL + MERCURIC OXIDE: EXPLOSION HAZARD.
LEAD: CORRODES.
LEAD PERCHLORATE: EXPLOSION HAZARD.
MAGNESIUM: VIOLENT REACTION.
MAGNESIUM (POWDERED): MIXTURES ARE CAPABLE OF DETONATION.
METALS: INCOMPATIBLE.
NICKEL: POSSIBLE IGNITION IN THE PRESENCE OF NICKEL CATALYST.

NITRIC ACID (CONCENTRATED): MIXTURES OF GREATER THAN 25% ACID MAY DECOMPOSE VIOLENTLY.
OXIDIZERS (STRONG): FIRE AND EXPLOSION HAZARD.
PERCHLORIC ACID: EXPLOSION HAZARD.
PHOSPHOROUS TRIOXIDE: POSSIBLE VIOLENT REACTION AND IGNITION.
PLASTICS, RUBBER, COATINGS: MAY BE ATTACKED.
POTASSIUM: POSSIBLE DANGEROUS REACTION.
POTASSIUM HYDROXIDE + CHLOROFORM: EXOTHERMIC REACTION.
POTASSIUM TERT-BUTOXIDE: FIRE AND EXPLOSION HAZARD.
SODIUM + CHLOROFORM: POSSIBLE EXPLOSION.
SODIUM HYPOCHLORITE: EXPLOSION HAZARD.
SODIUM METHOXIDE + CHLOROFORM: VIOLENT REACTION.
SULFURIC ACID: FIRE AND EXPLOSION HAZARD.
ZINC: EXPLOSION HAZARD.

DECOMPOSITION:
THERMAL DECOMPOSITION PRODUCTS MAY INCLUDE TOXIC OXIDES OF CARBON.

POLYMERIZATION:
HAZARDOUS POLYMERIZATION HAS NOT BEEN REPORTED TO OCCUR UNDER NORMAL TEMPERATURES AND PRESSURES.

STORAGE-DISPOSAL

OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN STORING OR DISPOSING OF THIS SUBSTANCE. FOR ASSISTANCE, CONTACT THE DISTRICT DIRECTOR OF THE ENVIRONMENTAL PROTECTION AGENCY.

****STORAGE****

STORE IN ACCORDANCE WITH 29 CFR 1910.106.

STORE AWAY FROM INCOMPATIBLE SUBSTANCES.

****DISPOSAL****

DISPOSAL MUST BE IN ACCORDANCE WITH STANDARDS APPLICABLE TO GENERATORS OF HAZARDOUS WASTE, 40 CFR 262. EPA HAZARDOUS WASTE NUMBER U154.

CONDITIONS TO AVOID

AVOID CONTACT WITH HEAT, SPARKS, FLAMES OR OTHER IGNITION SOURCES. VAPORS MAY BE EXPLOSIVE. MATERIAL IS POISONOUS; AVOID INHALATION OF VAPORS OR CONTACT WITH SKIN. DO NOT ALLOW MATERIAL TO CONTAMINATE WATER SOURCES.

SPILLS AND LEAKS

SOIL-RELEASE:
DIG HOLDING AREA SUCH AS LAGOON, POND OR PIT FOR CONTAINMENT.

DIKE FLOW OF SPILLED MATERIAL USING SOIL OR SANDBAGS OR FOAMED BARRIERS SUCH AS POLYURETHANE OR CONCRETE.

AIR-RELEASE:
APPLY WATER SPRAY TO KNOCK DOWN VAPORS.

WATER-SPILL:
ALLOW SPILLED MATERIAL TO AERATE.

LIMIT SPILL MOTION AND DISPERSION WITH NATURAL BARRIERS OR OIL SPILL CONTROL BOOMS.

USE SUCTION HOSES TO REMOVE TRAPPED SPILL MATERIAL.

OCCUPATIONAL-SPILL:
SHUT OFF IGNITION SOURCES. DO NOT TOUCH SPILLED MATERIAL. STOP LEAK IF YOU CAN DO IT WITHOUT RISK. USE WATER SPRAY TO REDUCE VAPORS. FOR SMALL SPILLS, TAKE UP WITH SAND OR OTHER ABSORBENT MATERIAL AND PLACE INTO CONTAINERS FOR LATER DISPOSAL. FOR LARGER SPILLS, DIKE FAR AHEAD OF SPILL FOR LATER DISPOSAL. NO SMOKING, FLAMES OR FLARES IN HAZARD AREA! KEEP UNNECESSARY PEOPLE AWAY; ISOLATE HAZARD AREA AND DENY ENTRY.

REPORTABLE QUANTITY (RQ): 5000 POUNDS
THE SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT (SARA) SECTION 304 REQUIRES THAT A RELEASE EQUAL TO OR GREATER THAN THE REPORTABLE QUANTITY FOR THIS SUBSTANCE BE IMMEDIATELY REPORTED TO THE LOCAL EMERGENCY PLANNING COMMITTEE AND THE STATE EMERGENCY RESPONSE COMMISSION (40 CFR 355.40). IF THE RELEASE OF THIS SUBSTANCE IS REPORTABLE UNDER CERCLA SECTION 103, THE NATIONAL RESPONSE CENTER MUST BE NOTIFIED IMMEDIATELY AT (800) 424-8802 OR (202) 426-2675 IN THE METROPOLITAN WASHINGTON, D.C. AREA (40 CFR 302.6).

PROTECTIVE EQUIPMENT SECTION

VENTILATION:
PROVIDE LOCAL EXHAUST OR PROCESS ENCLOSURE VENTILATION TO MEET THE PUBLISHED EXPOSURE LIMITS. VENTILATION EQUIPMENT MUST BE EXPLOSION-PROOF.

RESPIRATOR:
THE FOLLOWING RESPIRATORS AND MAXIMUM USE CONCENTRATIONS ARE RECOMMENDATIONS
BY THE U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, NIOSH POCKET GUIDE TO CHEMICAL HAZARDS; NIOSH CRITERIA DOCUMENTS OR BY THE U.S. DEPARTMENT OF LABOR, 29 CFR 1910 SUBPART Z.
THE SPECIFIC RESPIRATOR SELECTED MUST BE BASED ON CONTAMINATION LEVELS FOUND IN THE WORK PLACE, MUST NOT EXCEED THE WORKING LIMITS OF THE RESPIRATOR AND BE JOINTLY APPROVED BY THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH AND THE MINE SAFETY AND HEALTH ADMINISTRATION (NIOSH-MSHA).

METHYL ALCOHOL (METHANOL):

2000 PPM- ANY SUPPLIED-AIR RESPIRATOR.
ANY SELF-CONTAINED BREATHING APPARATUS.

5000 PPM- ANY SUPPLIED-AIR RESPIRATOR OPERATED IN A CONTINUOUS FLOW MODE.

10,000 PPM- ANY SELF-CONTAINED BREATHING APPARATUS WITH A FULL FACEPIECE.
ANY SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE.
ANY SUPPLIED-AIR RESPIRATOR WITH A TIGHT-FITTING FACEPIECE OPERATED IN A CONTINUOUS FLOW MODE.

25,000 PPM- ANY SUPPLIED-AIR RESPIRATOR WITH A FULL FACEPIECE AND OPERATED IN A PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

ESCAPE- ANY APPROPRIATE ESCAPE-TYPE SELF-CONTAINED BREATHING APPARATUS.

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

SELF-CONTAINED BREATHING APPARATUS WITH FULL FACEPIECE OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

SUPPLIED-AIR RESPIRATOR WITH FULL FACEPIECE AND OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE IN COMBINATION WITH AN AUXILIARY SELF-CONTAINED BREATHING APPARATUS OPERATED IN PRESSURE-DEMAND OR OTHER POSITIVE PRESSURE MODE.

CLOTHING:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE (IMPERVIOUS) CLOTHING AND EQUIPMENT TO PREVENT REPEATED OR PROLONGED SKIN CONTACT WITH THIS SUBSTANCE.

GLOVES:
EMPLOYEE MUST WEAR APPROPRIATE PROTECTIVE GLOVES TO PREVENT CONTACT WITH THIS SUBSTANCE.

EYE PROTECTION:
EMPLOYEE MUST WEAR SPLASH-PROOF OR DUST-RESISTANT SAFETY GOGGLES TO PREVENT EYE CONTACT WITH THIS SUBSTANCE.

EMERGENCY EYE WASH: WHERE THERE IS ANY POSSIBILITY THAT AN EMPLOYEE'S EYES MAY BE EXPOSED TO THIS SUBSTANCE, THE EMPLOYER SHOULD PROVIDE AN EYE WASH FOUNTAIN WITHIN THE IMMEDIATE WORK AREA FOR EMERGENCY USE.

AUTHORIZED BY- OCCUPATIONAL HEALTH SERVICES, INC.

CREATION DATE: 09/25/84 REVISION DATE: 10/09/90

APPENDIX J

CALCULATION OF MATRIX SPIKE PERCENT RECOVERY

CALCULATION OF MATRIX SPIKE PERCENT RECOVERY

The rationale and calculation of matrix spike recovery is as follows:

1. Calculate the change in concentration (Δ_s) expected due to the addition of the matrix (internal standard) spike.

2. Measure the concentration in the original solution (C_o) and the concentration in the spiked solution (C_s).

3. The percent recovery is the percent of the change in concentration relative to the expected change, or

$$\text{Percent recovery} = 100 \times \frac{(C_s - C_o)}{\Delta_s}$$

For example:

The original measure of the concentration of penta is 20 ppb = C_o

The spike is added to increase the concentration by 15 ppb = Δ_s

The measured concentration of the spiked sample is 34 ppb = Cs

$$\text{The percent recovery} = 100 \times \frac{(34 - 20)}{15} = 93\%$$

Therefore,

$$\text{Percent recovery} = \frac{\text{[sample + spike] - [unspiked sample]}}{\text{[spike added]}} \times 100$$

GLOSSARY OF IMMUNOASSAY TERMS

antibody -- a receptor protein (immunoglobulin) produced in response to an antigen that binds specifically to form an antigen-antibody complex.

antigen -- a large complex molecule that has a distinctive shape or functional group that can induce the formation of specific antibodies and can react to form an antigen-antibody complex.

chromogenic substrate -- compound that is converted to a colored end product when acted upon by an enzyme.

hapten -- a small antigenic molecule (e.g., PCP) that cannot induce an antibody response unless it is covalently bound to a carrier molecule forming a hapten-carrier conjugate.

immunoassay -- a physical assay based on the reversible interaction of a specific antibody with a target compound or compounds.

monoclonal antibody -- a homogeneous antibody population derived from one specific antibody-producing cell.

polyclonal antiserum -- a heterogeneous population of antibodies varying in antigenic specificity and affinity that is derived from several antibody-producing cells.