

Immunoassay Techniques in Environmental Analyses

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Abbreviations and Acronyms	15
Related Articles	16
References	16

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1 Introduction	1
2 Background and History	2
2.1 Overview of United States Environmental Protection Agency Regulatory Programs	2
2.2 Developmental History of Immunoassay Methods in United States Environmental Protection Agency Programs	3
3 Types of Immunoassay Technologies Employed in Environmental Programs	4
3.1 Enzyme-linked Immunosorbent Assay	4
3.2 Reporter Gene on a Human Cell Line	5
3.3 Immunosensors	6
3.4 Affinity Chromatography	6
3.5 Dissociation-enhancement Lanthanide Fluoroimmunoassay	6
4 Method Development	6
4.1 General Guidelines for Development of Screening Methods	6
4.2 Validation Criteria for Immunoassay Methods	7
5 Status of the United States Environmental Protection Agency Immunoassay Methods Development Program	8
5.1 Current Validated Methods	8
5.2 Methods under Development	9
5.3 List of Validated Immunoassay Test Products	11
6 Environmental Applications	11
6.1 Advantages and Disadvantages of Immunoassay Methods	11
6.2 Implementation Issues	12
6.3 Examples of Performance Based Measurement Systems	13
6.4 Applications as Screening Methods	13
6.5 Applications as Quantitative Methods	14
7 Future Directions	15
Acknowledgments	15

In the 1980s, several Program Offices of the United States Environmental Protection Agency (USEPA) investigated the use of immunoassay methods for environmental applications with limited success. Most of the problems were with method ruggedness, i.e. they worked well in clean spiked matrices, but not very well on real world samples. The situation changed significantly in January of 1992, when the Office of Solid Waste (OSW) received its first rugged immunoassay method (for pentachlorophenol (PCP)) that worked on field samples. Several more followed, and through the early and mid-1990s, the USEPA initiated use of immunoassay methodology in its hazardous waste program. The first group of methods incorporated into the OSW's methods compendium, Test Methods for Evaluating Solid Waste, or SW-846, between 1993 and 1995 were a group of 10 enzyme-linked immunosorbent assay (ELISA)-based screening methods for various environmental analyte classes. These methods were officially 'approved' by the USEPA in July, 1997. We provided a great deal of training on these methods to raise the comfort level of the environmental community for their use.

This chapter addresses several major topics including an overview of the USEPA's major Regulatory Programs; how analytical methods are used in Regulatory Programs; general guidelines for the development of screening methods; specific validation criteria for immunoassay methods; the current status of the USEPA immunoassay method development program; current and potential environmental applications for immunoassay technology; barriers to implementation of immunoassay methods and the steps being taken to overcome them; and future directions and new developments in immunoassay technology for regulatory programs.

The overall future of the technology for environmental monitoring and analysis looks very bright. It offers a cost effective way to generate reliable information upon which to base sound environmental decisions.

1 INTRODUCTION

Immunoassay technology has several attributes which make it a useful tool for environmental monitoring, e.g. selectivity, sensitivity, portability, and rapid turnaround time. Immunoassay kits can be tailored to target specific analytes or classes of analytes, thus eliminating the need for cleanup methods in most cases to remove interferences. They also have the capability of detecting

target analytes at very low levels, which are needed in many environmental applications. The portability of immunoassay test kits and speed of analysis allows for rapid analyses to be run on a site in the field. This capability can be especially useful in lowering the costs of cleanup projects because equipment does not have to lay idle while awaiting the results of laboratory analyses.

The USEPA has been looking at the potential use of immunoassay technology for environmental monitoring for several years. The early methods development efforts were unsuccessful because the immunoassay chemistry utilized in the methods was not sufficiently rugged for use on real world environmental matrices. The methods performed well on clean water matrices and spiked samples, but did not perform effectively on natural environmental samples. Because of this poor initial performance on real samples, USEPA Program Office interest in the technology declined.

In January 1992, EnSys, Inc. demonstrated a viable immunoassay test kit for PCP in both soil and water matrices to USEPA's OSW. Since that time, OSW has been working with several manufacturers to develop and validate a whole battery of immunoassay test kits both for individual analytes and for classes of analytes. The first series of immunoassay methods adopted by OSW for inclusion in its methods manual, *Test Methods for Evaluating Solid Waste*, (SW-846),⁽¹⁾ used ELISA as the technique of choice. Currently, OSW has issued more than fifteen immunoassay methods using several different techniques, which can be used for analyses performed under the Resource Conservation and Recovery Act (RCRA).⁽²⁾

2 BACKGROUND AND HISTORY

2.1 Overview of United States Environmental Protection Agency Regulatory Programs

2.1.1 Organization

The USEPA is not a single entity. It consists of several regulatory Program Offices which have the responsibility for implementing the major environmental laws passed by the US Congress, as well as several administrative and technical offices which support these regulatory Program Offices. These regulatory Program Offices include the Office of Solid Waste and Emergency Response (OSWER), the Office of Water (OW), the Office of Prevention, Pesticides and Toxic Substances (OPPTS), and the Office of Air and Radiation (OAR).

The various regulatory Program Offices were established at different times in response to Congressional mandates establishing the major environmental laws. These laws are very general in nature, and the USEPA

regulatory program offices were formed to set-up the specific regulations needed to administer and enforce these environmental laws. Approaches to regulatory requirements and philosophy vary greatly among the regulatory Program Offices, because of the significant differences in their areas of responsibility. For example, regulations governing air and water, dealing with single media, are necessarily different than those dealing with the management of solid and hazardous waste, which involve a wide variety of media.

2.1.2 Programmatic Regulatory Responsibilities

OSWER is responsible for administering the RCRA through its OSW and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund, through its Office of Emergency Response and Remediation (OERR). The OW administers the Clean Water Act (CWA) and the Safe Drinking Water Act (SDWA). The OPPTS administers the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), while the OAR administers the Clean Air Act (CAA). The Office of Administration and Resource Management (OARM) provides administrative support while the Office of Research and Development (ORD), along with several other non-regulatory program offices provide technical support to the regulatory Program Offices.

2.1.3 Regulatory Analytical Methods

The administration of these USEPA regulatory programs involves compliance monitoring, which utilizes a wide variety of analytical methods and techniques. This section of the chapter will focus on the similarities and differences only between the OSWER and OW monitoring programs.

The OSW regulates the management of solid and hazardous waste under RCRA. This includes regulations on generation, storage, and treatment of wastes and waste residuals from active management sites. Most RCRA applications involve site-specific analyses for compliance with permits or other regulations, unlike the general maximum contaminant level (MCL) requirements common to compliance with drinking water regulations. Since RCRA applications encompass a wide variety of media, including, soils, solids, sludges, organic liquids, water, stack emissions, and ambient air, OSW has historically used the performance based measurement system (PBMS) approach to sampling and analysis. This allows for the needed flexibility in matrix-specific and project-specific methods selection for RCRA applications.

The OSW Methods Team publishes a manual of analytical methods applicable for use in the RCRA

Program, *Test Methods for Evaluating Solid Waste*, SW-846. SW-846 functions primarily as a guidance document. In most cases under RCRA, analyses may be performed using either SW-846 methods or any other method that may be appropriate, since RCRA regulations usually only specify what the analytical requirements are, and not specifically how to do them. Whichever methods are selected, the analyst must demonstrate their applicability for their intended use. This can be done by using a two-tiered demonstration of proficiency: 1) the analyst must demonstrate the ability to perform the method and obtain acceptable results in a 'clean' matrix, such as reagent water or Ottawa sand; and 2) the analyst must demonstrate that the method will give acceptable performance in the actual matrix of concern.

However, there are a very few instances in the RCRA regulations where SW-846 analytical methods, in general, must be used to characterize waste. Because of these few cases which require the use of SW-846 methods, all new and revised methods which are included in SW-846 must be published as regulations. OSW is currently working on a regulation under the 'Reinvention of Government' guidelines to eliminate this requirement and to publish SW-846 methods as guidance as was the original intent of the manual. Methods which will not become guidance are the 'method-defined parameters', or methods which directly define regulatory requirements, e.g. flash point, corrosivity.

The OERR, on the other hand, deals with the cleanup of abandoned waste sites under CERCLA (Superfund). Most Superfund analyses are performed using the same multimedia PBMS approach as is used for RCRA. Some Superfund analyses are performed under specific contracts which can use any method deemed appropriate for the application. However, when Superfund methods are written into their contract format, they are very detailed and must be performed as written. Changes or modifications are generally not permitted.

The OW, for compliance with both the CWA and SDWA, is required to publish lists of both regulated and unregulated target analytes and to publish approved methods for the analyses of these analytes. Use of alternative methods is not permitted, unless a method equivalency petition is requested by a petitioner and granted by USEPA or other designated authority.

Thus, of the four major USEPA Program Offices for which substantial numbers of analyses are performed, RCRA offers the most flexibility as to choice and application of analytical methods. RCRA only specifies what the analytical requirements are, and leaves the decision as to how the analyses are to be performed to the analyst.

2.2 Developmental History of Immunoassay Methods in United States Environmental Protection Agency Programs

While several USEPA Program Offices investigated the potential applicability of immunoassay methods to their programs, OSW is the first USEPA Program Office to formally incorporate these techniques into its methods program. OSW has taken the Agency lead in developing new immunoassay techniques for its own methods program and is actively working with other USEPA Program Offices to assist them in developing immunoassay methods for their programs.

OSW began evaluation of its first immunoassay method (for PCP) in January 1992, followed by three others in rapid succession. By July 1995, OSW had completed validation of a general overview method and ten individual immunoassay methods (with more than 25 different kits) utilizing ELISA, and published them in the proposed Update III of SW-846. For a USEPA regulatory Program Office, this is very rapid progress. These immunoassay methods covered the analysis of PCP, polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), petroleum hydrocarbons, pesticides and explosives. See Table 1 for a list of these methods. OSW formally approved the first immunoassay method, for PCP, as part of a January 1994, regulation. The rest of the methods in Table 1 were formally approved as part of Update III to SW-846 in June, 1997. All of these methods can be used for any RCRA application for which they can be demonstrated to work.

Between late 1995 and late 1997, the environmental immunoassay industry fell on hard times due to severe cutbacks in remediation projects for which there was a major potential market for use of these environmental immunoassays. This resulted in a consolidation of the

Table 1 Immunoassay methods in update III of SW-846

Method 4000: Immunoassay ⁽³⁻¹⁰⁾
Method 4010: PCP in Water and Soils by Immunoassay ⁽¹¹⁻¹⁴⁾
Method 4015: 2,4-D in Water and Soils by Immunoassay ⁽¹⁵⁻¹⁷⁾
Method 4020: PCBs in Soil by Immunoassay ⁽¹⁸⁻²⁶⁾
Method 4030: TPH in Soil by Immunoassay ⁽²⁷⁻²⁹⁾
Method 4035: Soil Screening for PAHs by Immunoassay ^(30-32,80)
Method 4040: Toxaphene in Soils by Immunoassay ^(33,34)
Method 4041: Chlordane in Soils by Immunoassay ⁽³⁵⁾
Method 4042: DDT in Soils by Immunoassay ⁽³⁶⁾
Method 4050: TNT Explosives in Water and Soils by Immunoassay ⁽³⁷⁻⁴⁰⁾
Method 4051: RDX Explosives in Water and Soils by Immunoassay ^(40,41)

2,4-D, 2,4-dichlorophenoxyacetic acid; TPH, total petroleum hydrocarbons; TNT, 2,4,6-trinitrotoluene; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine.

four major manufacturers into a single entity. While the industry was busy sorting itself out during this period, very little was happening in the area of new product and methods development. However, by the end of 1997, several new manufacturers began operating and several new products have either come on the market or are in the final stages of testing, including second generation ELISA kits for PAHs and PCBs, the first immunoassay method (ELISA for mercury) for a metal, immunosensors for explosives, and, finally a series of screening methods for dioxins and coplanar PCBs using a variety of techniques.

Some of the other USEPA Program Offices are also looking at immunoassay methods to address some of their analytical requirements. OPPTS is considering using immunoassay methods in its Pesticide Registration Program. OW is beginning to look at using the technology in both the Drinking Water and Wastewater Programs. However, they may have to revise some of their regulations to allow for the use of 'less than' values in reporting MCLs or look toward developing quantitative immunoassays.

3 TYPES OF IMMUNOASSAY TECHNOLOGIES EMPLOYED IN ENVIRONMENTAL PROGRAMS

3.1 Enzyme-linked Immunosorbent Assay

ELISA⁽³⁻¹⁰⁾ is the most commonly used immunoassay technique for environmental analysis. The immunoassay test products available will often vary in both format and chemistry. The characteristics of a specific product are described in the package insert provided by the manufacturer. This summary is, therefore, general in scope, and is intended to provide a general description of the more common elements of these methods.

Immunoassay test products use an antibody molecule to detect and quantitate a substance in a test sample. These testing products combine the specific binding characteristics of an antibody molecule with a detection chemistry that produces a detectable response used for interpretation. In general, antibody molecules specific for the method's intended target are provided at a predefined concentration. A reporter (i.e. signal generating) reagent, composed of the target compound conjugated to a signal producing compound or molecule (e.g. enzymes, chromophores, fluorophores, luminescent compounds, etc.), is also provided. The concentration, affinity, and specificity of the products' antibody influences performance, as does the chemistry of the reporter reagent.

The reporter reagent and antibody molecules of a given product are binding partners, and form a complex in solution. The addition of a positive sample containing the

target substance to this solution results in a competitive binding reaction between the target analyte and the reporter reagent for the antibody sites. The antibody concentration, and therefore binding capacity, is limited to prevent the simultaneous binding of both the reporter and target molecules. The concentration of reporter reagent that can bind to the antibody is inversely proportional to the concentration of substance in the test sample.

Immunoassay methods may be heterogeneous (i.e. requiring a wash or separation step), or homogeneous (i.e. not requiring a separation step). In commonly available heterogeneous testing products, the antibody is immobilized to a solid support such as a disposable test tube, and the bound reporter reagent will be retained after removing the unbound contents of the tube by washing. Therefore, a negative sample results in the retention of more reporter molecules than a positive sample. The analysis of a standard containing a known concentration results in the immobilization of a proportional concentration of reporter reagent. A positive sample (i.e. containing a higher concentration than the standard) results in the immobilization of fewer reporter molecules than the standard, and a negative sample (i.e. containing less than the standard) will immobilize more.

A chemistry of the detection of the immobilized reporter is used for interpretation of results. The reporter molecule may be a conjugate of the target molecule and a directly detectable chromophore, fluorophore, or other specie, or conjugated to an enzyme that will act upon a substrate to produce the detectable response. Immunoassay testing products have a quantitative basis, and will produce a signal that is dependant on the concentration of analyte present in the sample. For environmental immunoassay methods, the signal produced is exponentially related to the concentration of the compounds present. Many immunoassay methods use enzymes to develop a chromogenic response, and are termed enzyme immunoassays. Assays that generate a chromogenic response are analyzed photometrically, and use the principles of Beer's law ($\text{absorbance} = \text{extinction coefficient} \times \text{concentration} \times \text{pathlength}$) to determine the concentration of analyte in a sample.

Immunoassay methods can provide quantitative data when configured with a series of reference standards that are analyzed and used to construct a standard curve. The signal generated from the analysis of a test sample is used to determine concentration by interpolation from the standard curve. Alternatively, these testing products can be configured to determine if a sample is positive or negative relative to a single standard.

Individual immunoassay testing products are reviewed and accepted by the USEPA OSW for the detection of sample analytes in specified matrices. A variety of testing products, produced by several different developers, may

be available for the same compound(s) and matrices. Each of these methods have been formulated using independently developed reagents that may result in significantly different performance characteristics and limitations.

The performance of the immunoassay testing products ultimately relates to the characteristics of the antibody, reporter molecule, and sample processing chemistry. The dose-response characteristics of a method, the position of the standard relative to the claimed action level, and the stated cross-reactivity characteristics of the selected test product, provide relevant information regarding the performance and recognition profile of the selected test product.

The precision, and ultimately the sensitivity of an immunoassay method, is a function of the signal-to-noise characteristics of its dose-response curve, and its operational consistency. Methods having a high slope and low non-specific signal generation produce the most sensitive and precise methods. Signal imprecision applied to a dose-response curve having a shallow slope exhibits proportionally greater imprecision in the calculated concentration than would a method having a steeper slope. In an action level testing product, this would cause the reference standard to be positioned further from the action level, increasing the incidence of false positive results. Similarly, a method having less non-specific signal generation (higher signal-to-noise ratio) will be more sensitive and precise when other characteristics (i.e. dose-response slope) are held constant.

Immunoassay methods are used to detect contamination at a specific concentration below the claimed detection level for the test product. For example, an immunoassay used to detect PCB contamination in soil at 1 ppm will include a standard preparation containing less than 1 ppm. The reference preparation concentration is positioned to minimize the incidence of false negative results at the claimed detection level. For remediation and monitoring applications, where action levels of interest are defined, immunoassay methods should exhibit a negligible incidence of false negative results, and minimal false positives.

For a single point action level test, the concentration of analyte relative to the action level is selected by the developer, and is influenced by the precision (i.e. intra-assay, inter-person, inter-lot, inter-day, etc.), sample matrix interferences and other performance characteristics and limitations of the basic method. The concentration of analyte in the reference materials should be less than, but close to, the claimed action level. The concentration selected for the standard defines the concentration that will produce a 50% incidence of false positive results by the test product. While this issue is one representing limited liability to the operator, it is a practical issue that often requires attention. An immunoassay method for the

detection of 1 ppm of PCB using a standard containing 0.8 ppm of PCB will experience a 50% false positive incidence in samples containing 0.8 ppm of PCB, and some incidence of false positive results in a sample containing between 0.8 and 1 ppm. A similar immunoassay that uses a standard containing 0.4 ppm will experience a 50% false positive incidence in samples containing 0.4 ppm of PCB, and some incidence of false positive results in a sample containing between 0.4 and 1 ppm. The closer the standard concentration is to the action level, the better the overall performance.

Cross reactivity characteristics illustrate the specificity of the underlying immunochemistry. The antibody molecules used by a test product bind to a target compound and then participate in the process of generating the signal used for interpretation. Antibody molecules bind by conformational complementarity. These molecules can be exquisitely specific, and can differentiate subtle differences in the structure of a compound. The binding characteristics of reagents in different test products can vary, and influence the recognition profile and incidence of false results obtained by the method. Immunoassay methods should detect the target analytes claimed by the test product and exhibit limited recognition for compounds and substances not specified.

3.2 Reporter Gene on a Human Cell Line

The reporter gene system⁽⁴²⁻⁵⁰⁾ utilizes a human cell line (101L) into which a plasmid containing a human CYP1A1 promoter and 5'-flanking sequences fused to a reporter gene, firefly luciferase, have been stably integrated. In the presence of CYP1A1-inducing compounds, the enzyme luciferase is produced, and its reaction with luciferin can be detected by measuring relative light units (RLUs) in a luminometer. CYP1A1-inducing environmental contaminants include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar PCB congeners, and high molecular weight PAHs.

To quantify the inducing compounds in the sample, the mean response, in RLUs, of the three sample replicates is divided by the mean response of three replicates of a solvent blank, yielding a 'fold induction', which is a measure of the increase of the sample response over the background response. Fold induction may be converted to toxic equivalents (TEQ) for PCBs and PCDDs/PCDFs, or benzo[*a*]pyrene equivalents (B[*a*]PEqs) for PAHs, based on the fold induction responses to standards containing a mixture of PCDDs/PCDFs, or benzo[*a*]pyrene, respectively.

This method can be used to estimate the concentrations of PAHs and/or coplanar PCBs in soils over a range from 0.1 to over 100 mg kg⁻¹. In addition, the presence

of PCDDs/PCDFs in the extract will be indicated at concentrations ranging from 100 ng kg^{-1} to over 1 mg kg^{-1} . These sensitivity estimates are based on the extraction of 40-g solid samples and evaporation of the extract to 1 mL, with the application of $20 \mu\text{L}$ to one million cells covered by 2 mL of medium. The sensitivity of the method for water samples is approximately 25 times greater, assuming that a 1-L water sample is extracted and the extract is concentrated to the same 1-mL volume (e.g. $4 \mu\text{g L}^{-1}$ to 4 mg L^{-1} for PAHs and PCBs, and 4 ng L^{-1} to $40 \mu\text{g L}^{-1}$ for PCDDs/PCDFs).

The method contains an optional procedure in which exposures are conducted over two specific time periods (6 and 16 hours). This option allows the test to distinguish between PAHs in the samples and chlorinated compounds, since the PAHs reach maximum induction at 6 hours, while the peak in induction from chlorinated planar compounds (PCBs, PCDDs/PCDFs) is not until 16 hours.

This method is a screening procedure, and depending on project needs. A subset of the samples should be confirmed using quantitative analytical techniques.

3.3 Immunosensors

Two types of immunosensor techniques have been evaluated at this time,^(51–68) both of them for TNT and RDX explosives. The first, a flow cell technique is based on performing a fluorescent displacement immunoassay on a membrane or other solid support in flow. Antibodies immobilized on a solid support (i.e. membrane) are saturated with a fluorescent analog of either TNT or RDX. A test sample is injected into the flow stream. If the appropriate analyte (TNT or RDX) is present in the test sample, it will displace a proportional amount of fluorescent analog. The displaced analog is detected downstream. The fluorescent signal is proportional to the concentration of the analyte in the test sample. This technique can be used as a screening tool to determine if TNT or RDX is present above a critical limit or for quantitation of the explosive in the test sample. To quantitate, the area of the fluorescent peak is compared to standards tested pre and/or post test sample. Results obtained with this method can be used to identify samples with TNT and RDX concentrations between 10 and 1200 ppb.

The second, a fiberoptic technique, is based on performing a competitive fluorescent immunoassay on the surface of a fiber optic probe. A fluorescent analog of either TNT or RDX is added to the test sample. The sample is then passed over optical probes which have antibodies immobilized on the surface which are specific for either TNT or RDX. A decrease in maximum fluorescence proportional to the concentration of TNT or RDX in the sample is observed. This technique can

be used as a screening tool to determine if TNT or RDX is present above a critical limit or for quantitation of the explosive in the test sample. To quantitate, the percent inhibition is compared to a standard curve. Results obtained with this method can be used to identify samples with TNT and RDX concentrations between 5 and 200 ppb.

3.4 Affinity Chromatography

Affinity chromatography is a potential sample preparation technique whereby the selective nature of immunoassay methods, particularly ELISA, can be used to extract specific compounds or compound classes out of a difficult matrix. The bound analytes can then be desorbed and analyzed by an appropriate chromatographic or other determinative technique, e.g. high-performance liquid chromatography (HPLC).

3.5 Dissociation-enhancement Lanthanide Fluoroimmunoassay

The DELFIA (dissociation-enhancement lanthanide fluoroimmunoassay) methodology was developed by scientists at EG&G Wallac, Turku, Finland and the Department of Molecular Endocrinology of Middlesex Hospital, London.⁽⁸⁴⁾ This method is clinically proven and is highly reliable and adaptive. The DELFIA system is based on time-resolved fluorometry of lanthanide compounds such as europium. Lanthanide ions exhibit a unique fluorescence that is characterized by narrowband emission lines, a long decay time, and large Stokes shift. The specific fluorescence of the lanthanide label is measured after a certain time delay following an activation pulse allowing all of the nonspecific background to expire. The DELFIA system and instrumentation allows the detection of four different toxicants in one sample by using four different lanthanide labels each having a distinctive fluorescence spectrum.

4 METHOD DEVELOPMENT

4.1 General Guidelines for Development of Screening Methods

The primary applicability that we, in the RCRA Program, see for immunoassay methods is for quantitative screening purposes.⁽⁶⁹⁾ By quantitative screening, we mean setting a quantitative action level (usually the regulatory action level), where a positive response means that the analyte is present at or above the action level, and a negative response tells us that the analyte is either absent or present below the level of regulatory concern. Analyses can be run at multiple action levels giving a useful range of concentrations for specific target analytes.

For example, if we are mapping a site contaminated with PCBs to determine the extent to which it needs to be cleaned up, knowing where PCB levels are <10 ppm, between 10 and 100 ppm, and >100 ppm can be useful in planning and expediting the cleanup.

The OSW Methods Team provides a guidance document (available on-line at <http://www.epa.gov/SW-846>) to potential developers of screening methods providing guidance on what general validation criteria should be applied to a screening method that will potentially be included in SW-846. While screening procedures need not be fully quantitative, they should measure the presence or absence of target analytes at or below regulatory action levels. Therefore, initial demonstration of method performance involves measuring the percentage of false negatives and false positives generated using the procedure for a single sample matrix. Data should be submitted for split samples analyzed using the developer's technique and an appropriate SW-846 quantitative method. A candidate procedure should ideally produce no false negatives. Definition of a false negative is a negative response for a sample that contains the stated detection level of the target analyte(s). A candidate procedure should produce no more than 10% false positives. Definition of a false positive is a positive response for a sample that does not contain analytes at the detection level. Between 20 and 50 samples spiked at the detection level should be tested to establish the percentage of false positives. Between 20 and 50 samples spiked at the detection level should also be tested to establish the percentage of false negatives. It is recommended that a sufficient volume of each spiked sample be prepared to complete each test with one lot of material. Sufficient randomly selected aliquots of each spiked matrix should be analyzed by appropriate SW-846 methods to demonstrate sample homogeneity and to characterize the sample in terms of target analytes and potential interferences.

A separate study should also be conducted to establish the effect of non-target interferences. A screening procedure should produce no more than 10% false positives for a set of 20 samples that contains a 100 fold excess of interferences. Positive interferences should be selected that are chemically related to the target analytes and are environmentally relevant. Negative interferences (i.e. masking agents) should also be investigated whenever they are suspected.

Developers should also analyze three different types of samples to provide matrix-specific performance data. These samples should either be characterized reference materials or spiked matrices containing known amounts of target analytes. In either case, bulk samples should be carefully homogenized to reduce sub-sampling errors. The sample matrices should be selected to represent what is regulated under RCRA (e.g. soil, oily waste or

wastewaters), not to provide the best performance data. Blanks should be analyzed with each set of samples.

Matrix-specific performance data, including detection limits and dynamic range, are gathered by analyzing ten replicate aliquots of three different sample matrices spiked at two concentrations. If spiked samples are used, suggested spiking levels are the matrix-specific detection limit and 50 times the detection limit. Positive or negative results should be reported for the low concentration samples. Results for high concentration samples should be reported as either semi-quantitative results or as positive/negative with the dilution factor used for the samples. As an alternative to establishing matrix-specific detection limits, specific spiking concentrations are provided for selected target analytes in the guidance document. The low values are normal reporting limits for routine analyses and the high value is 50 times the low concentrations. The Methods Team recognizes that it may not be appropriate to spike all of the target analytes listed within a chemical class.

If the developer has field data, the Methods Team would welcome the opportunity to compare the results obtained using the screening procedure with sample concentrations determined in a laboratory using SW-846 or other appropriate methods.

To summarize, the Methods Team does not require an unreasonable body of data for the initial evaluation of new techniques. Data will need to be submitted on the percentage of false negatives, percentage of false positives, sensitivity to method interferences, and matrix-specific performance data. In addition to these data, the developer should also provide a description of the procedure and a copy of any instructions provided with the test kits.

4.2 Validation Criteria for Immunoassay Methods

4.2.1 Screening Methods

In addition to the guidelines for developing screening methods in general, OSW, based on its own experience, has generated some validation criteria specifically applicable to immunoassay methods.^(70,71) These validation criteria, based on the United States Food and Drug Administration (USFDA) 510 K guidelines, are required to be submitted to OSW for review for all immunoassay test kits, whether the kits are to be the basis for a new method or as an alternative kit being added to existing methods. The data needed for validation of immunoassay methods that will be included directly in the method is as follows:

1. cross reactivity with similar analytes;
2. cross reactivity with dissimilar analytes which may be reasonably expected to be found at waste sites;
3. false negative/false positive rates;

4. extraction efficiency (for soil test kits);
5. performance data on spiked samples in environmental matrices validated against standard SW-846 analytical methods;
6. performance data on actual environmental field samples validated against standard SW-846 analytical methods.

Since interferences can be a major problem in environmental analyses, it is important to demonstrate that the analytes of concern can be identified in the presence of similar analytes or dissimilar analytes which may be present in environmental samples. In many instances, substantial cross reactivity with other analytes is a desirable situation. Examples of desirable cross reactivity include sensitivity to esters of 2,4-D as well as the 2,4-D, and for other 3-, 4-, and 5-membered PAHs when testing for phenanthrene in a PAH screening method.

The false negative/false positive rate for a particular immunoassay kit is very important. OSW screening methods are designed to generate 0% false negatives and up to 10% false positives at the regulatory action level. Slightly higher false positive rates are tolerable, e.g. up to 25%. High false positive rates, i.e. >25%, negate the cost effectiveness of the technique because of the excessive numbers of confirmatory tests that would need to be performed. High false negative rates, i.e. >5% at the regulatory action level eliminate the potential use of the method for regulatory purposes.

The extraction efficiency data are important for setting the appropriate action level for a soil analysis. Recoveries are the primary determining factor for making sure that the analyte of concern can be detected at the regulatory action level and for minimizing false negative/false positive rates. Extraction efficiencies need not be quantitative, because of the sensitivity of the antibodies used. However, they must be consistent and reproducible.

The performance data generated from environmental samples spiked with the target analytes give a good indication as to whether or not an immunoassay method will work. However, the performance generated in the field on real environmental samples is the key determining factor on whether or not the immunoassay method is sufficiently rugged to be included in SW-846 as an analytical method.

Additional data that OSW requests, but does not include in the method and treats as confidential business information (CBI), includes dosage curves and the manufacturer's internal validation and quality control criteria. The slope of the dosage curve can be a good indication of whether or not an immunoassay method will exhibit a high rate of false positives.

Up to this time, all of the immunoassay test kits (>25) that the OSW has evaluated have been extensively tested

and validated by the manufacturers. USEPA validation has primarily consisted of confirmation of the manufacturers' results and performing some additional testing on well-characterized environmental samples, which are more easily available to USEPA regional laboratories.

4.2.2 Quantitative Methods

OSW has issued a guidance document for the validation of quantitative methods, "Guidance for Methods Development and Methods Validation for the RCRA Program",⁽⁶⁹⁾ describing the key elements that need to be met from the Proof of Concept stage through single and multilaboratory validation. The document is also available on the Internet from the OSW Methods Team Homepage at "<http://www.epa.gov/SW-846>". Quantitative immunoassay methods would be validated using the same criteria as any other quantitative method.

4.2.3 Revalidation Issues

Based on the successful incorporation of ten immunoassay methods involving more than 25 validated individual test products, the issue has come up as to what type of revalidation of a previously validated method is necessary if that method is changed. Once again, we defer to the USFDA for guidance on this issue. The guidelines that will be included in the revised Immunoassay Screening Methods Development Guidance Document are that for non-substantive changes, i.e. changes that do not affect the basic chemistry of the method, no revalidation will be necessary. An example of this type of change would be change of an inert washing solution in an ELISA kit.

If the manufacturer decided to change the format of the product, e.g. from a tube reaction to a home pregnancy test kit type format without changing the chemistry, the manufacturer would need to do a partial revalidation. This partial revalidation would need to be sufficient to demonstrate that the format change has not adversely affected or changed the chemistry and that the new kit gives the same results as the old one on split samples.

If the manufacturer changes the chemistry in an existing product, this would be considered as a new product and would require a new validation.

5 STATUS OF THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY IMMUNOASSAY METHODS DEVELOPMENT PROGRAM

5.1 Current Validated Methods

The first eleven immunoassay methods (see Table 1) were formally approved for incorporation into SW-846

by regulation in June, 1997. The generic Immunoassay method (Method 4000) and the ten individual ELISA methods may be used for any analytical application for which they can be demonstrated to be appropriate. No regulatory barriers remain to prevent the use of these methods.

The five new methods included in Table 2 have been formally validated and accepted by OSW's Technical Workgroup. However, until the regulations removing the requirements to adopt new methods through regulatory action are removed (expected to be completed by the end of 2000), the use of these methods may be limited by some regulatory restrictions. However, for most of the applications for which they are intended to be used, there are no actual regulatory barriers, only perceptions.

Method 4425, developed by Columbia Analytical Services (CAS),⁽⁴²⁻⁵⁰⁾ is a gross screening method using a reporter gene on a human liver cell line to screen for PAHs, PCBs and dioxins. The method can distinguish between PAHs and chlorinated compounds by differences in reaction time. The method can give useful semiquantitative information only on well characterized sites in the monitoring mode.

Method 4500, developed by BioNebraska,^(72,73,92) is the first USEPA immunoassay method for a metal, mercury. It provides semiquantitative screening data for mercury extracted from soils with acid and followed by a colorimetric determination using ELISA.

Methods 4655 and 4656 are immunosensor probe methods for TNT and RDX explosives developed by the Naval Research Laboratory (NRL)^(51-68,81,82) using previously developed ELISA antibodies used in the earlier test kit methods (see Methods 4050 and 4051 in Table 1) and detection using a flow cell technique based on performing a fluorescent displacement immunoassay on a membrane or other solid support in flow or a fiberoptic technique based on performing a competitive fluorescent immunoassay on the surface of a fiber optic probe.

Table 2 Next generation of methods validated for use in RCRA programs

Method 4425: Screening Extracts of Environmental Samples for Planar Organic Compounds (PAHs, PCBs, Dioxins/Furans) by a Reporter Gene on a Human Cell Line ^a
Method 4500: Mercury in Soil by Immunoassay ^a
Method 4655: Explosives Analysis in Soil and Water Using Environmental Immunosensors ^a
Method 4656: Explosives Analysis in Soil and Water Using Fiber-optic Immunosensors ^a
Method 4670: Triazines as Atrazine in Water by Immunoassay ^a

^a Draft methods. Can be used for applications where the use of promulgated SW-846 methods is not necessary.

Method 4670^(74-78,80) developed by Ohmicron (SDI, Inc.) is a competitive ELISA immunoassay method for the quantitative determination of triazine herbicides as atrazine in drinking water. This method will be discussed in more detail in section 6.5.

Copies of the methods in Table 2 are available now or will be shortly on the OSW Methods Team Homepage at "http://www.epa.gov/SW-846".

5.2 Methods under Development

The six methods listed in Table 3 are in various stages of development. Methods 4025 for dioxin and 4026 for coplanar PCBs are ELISA methods from Cape Technologies and should have their validations completed by the end of 1999. The validation of the DELFIA method for dioxin TEQ, Method 4430, from Hybrizyme should also be completed around the same time. The other three methods, Method 36xx for affinity chromatography, Method 4016 for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and Method 4060 for TCE are all only in the beginning stages of development at this time.

Dioxin is a general term that describes a group of highly toxic chemicals that are extremely persistent in the environment. These compounds have been of major interest to immunoassay method developers. There are 210 different dioxin and furan congeners, 17 of which have been determined to be toxic. Each toxic congener has been assigned a toxic equivalency factor (TEF) which expresses how biologically active it is as compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic congener. Studies have demonstrated that the toxicity of the individual congeners is additive. The additive effects of these compounds are expressed as total TEQ in comparison to TCDD.^(85,86)

The scientific community has now identified a common pathway responsible for most, if not all, of the harmful effects associated with exposure to dioxin and related compounds. The interaction of dioxin-like compounds with a cellular protein known as the "Aryl hydrocarbon receptor" or "Ah receptor" represents the first step in a series of events that ultimately alters normal physiological processes.⁽⁸⁷⁾ Exposure to dioxin-like molecules has been

Table 3 New methods under development

Method 36xx: Affinity Chromatography
Method 4016: 2,4,5-T in Water by Immunoassay
Method 4025: Dioxin in Water and Soil by Immunoassay
Method 4026: Coplanar PCBs in Water and Soil by Immunoassay
Method 4060: TCE in Soil by Immunoassay
Method 4430: Dioxin TEQ in Water and Soil by DELFIA

TCE, trichloroethylene.

linked to a variety of illnesses including cancer, harmful reproductive and developmental effects, immunotoxicity, diabetes, and endometriosis.⁽⁸⁸⁾

Discovery of the Ah receptor provided a key to understanding the molecular mechanism of dioxin toxicity. The Ah receptor resembles the broad category of steroid hormone receptors and like those receptors, it regulates gene transcription. The receptor is capable of a high degree of structural discrimination between molecules and of transducing signals at very low concentrations. The toxicity of the 17 dioxins and furans results from changes in the expression of critical genes following binding and activation of the Ah receptor. Therefore, biological systems respond to the cumulative exposure of Ah receptor-mediated chemicals rather than to the exposure to any single dioxin-like compound, thus providing the molecular basis for the empirically derived TEF and TEQ value system.⁽⁸⁹⁾

Several different approaches have been used for screening of toxic dioxins and furans by immunoassay. These include the reporter gene approach (Method 4425) described in the previous section, ELISA (Method 4025) and DELFIA (Method 4430).

Cape Technologies' Method 4025 (ELISA) is a screening method that measures TEQ of dioxins and furans in

soil and water extracts. It does involve a detailed sample preparation procedure, unlike the test kits for simpler target analytes. Therefore it is more suitable for laboratory use than for field use. The method is readily sensitive to 500 ppt TEQ in soils.⁽⁹⁰⁾

Hybrizyme's dioxin assay (Method 4430) is based upon a recombinant version of the Ah receptor gene which is used in the assay to accurately measure dioxin-like compounds. The DNA sequence adjacent to the Ah receptor gene has been modified so that the recombinant protein can be manufactured in large quantities and easily incorporated into an immunoassay format. Accordingly, the assay embodies 'nature's perfect device' for detecting the presence of these compounds and provides the user with a 'risk-based' approach to TEQ analysis. In conjunction with the Ah receptor-based assay system, Hybrizyme has developed sample processing procedures that will allow the test to selectively detect dioxins and furans, co-planar PCBs, or carcinogenic PAHs such as benzo[*a*]pyrene.

The Method 4430 dioxin assay is noncompetitive by design. Almost all immunoassays that detect small molecules are competitive in nature. The sensitivity of noncompetitive assays increases with lower

Table 4 Immunoassay test products validated by OSW

Method # Analyte	Manufacturer						
	Beacon (SDI)	BioNebraska	Columbia Anal. Serv.	EnSys (SDI)	Millipore (SDI)	Ohmicron (SDI)	SDI
4010 PCP				Soil & Water	Soil & Water	Soil	
4015 2,4-D					Water & Soil	Water & Soil	
4020 PCB	Soil			Soil Oil	Soil	Soil	Soil
4030 TPH				Soil	Soil Soil (SCHC)	Soil (SCHC)	
4035 PAH	Soil (A/CPAH)			Soil (APAH)	Soil (APAH)	Soil (APAH) Soil (CPAH)	
4040 Toxaphene					Soil		
4041 Chlordane					Soil		
4042 DDT							
4050 TNT					Soil	Soil	Soil
4051 RDX							Soil
4425 PAH, PCB, Dioxins			Soil Gross Screen				
4500 Mercury		Soil					
4670 Triazines						Water	

APAH, anthracene polynuclear aromatic hydrocarbons; CPAH, carcinogenic polynuclear aromatic hydrocarbons; SCHC, short-chain hydrocarbons.

immunoreactant concentrations since, at low concentrations, a variation in the number of competing molecules has a larger impact on the interaction with the labeled species. However, according to the law of mass action, the low antigen and antibody concentrations considerably reduce the rate of complex formation and, at very low concentrations, the accuracy tends to be poor. As a non-competitive assay, the Ah receptor test will utilize a large excess of receptor to obtain a maximum signal for the compound being tested. Even at very low concentrations of molecules, a high fraction will react if the receptor is added in excess. Although not practically achievable, the theoretical limit of the detection for this system is one molecule.⁽⁹¹⁾

The Hybrizyme DELFIA assay provides an ultra-sensitive measurement system for detecting dioxin-like molecules that interact with the Ah receptor. By utilizing molecular cloning methods and clinical instrumentation, the method provides the analytical laboratory with a product containing built-in QA/QC routines, retained standard curves, LIMS compatible data-reduction, and the ease-of-use demanded in state-of-the-art clinical laboratories.

Cape Technologies has also developed an ELISA-based immunoassay for coplanar PCBs (Method 4026)⁽⁹³⁾ similar to its dioxin immunoassay (Method 4025). The method is sensitive to the 14 dioxin-like PCB congeners and is in the process of field testing to complete the final validation for inclusion in SW-846.

5.3 List of Validated Immunoassay Test Products

Table 4 provides a list of individual immunoassay test products that have been validated by OSW and were commercially available. Due to the merger of the four major manufacturers in 1997 and a reorganization of product lines, some of the listed ELISA kits may have been discontinued.

6 ENVIRONMENTAL APPLICATIONS

6.1 Advantages and Disadvantages of Immunoassay Methods

The use of immunoassay methods provides many advantages to the analyst in solving environmental problems. Some of the significant advantages derived from using this technology include selectivity, sensitivity, portability, rapid turnaround time, improved data quality, and overall cost effectiveness. Immunoassay methods by their very nature and design are very selective in their scope of applicability, i.e. they are designed to be very specific for the analysis of either an individual compound, e.g. 2,4-D or toxaphene, or an individual class of compounds, e.g.

PAHs or PCBs. Compounds other than the target analytes which may be present on a potential site do not react under the conditions and reagents used for the particular methods.

Sensitivity is another advantage of immunoassay technology. Many target analytes can be detected in the ppm range or low ppb range, if necessary. Many commercial products can be obtained or prepared to operate in several ranges, e.g. 1, 10 and 100 ppm, allowing for useful site mapping to determine which areas of a contaminated site are in need of cleanup and which are not. Some kits can operate both at the low ppb range and with appropriate dilution in the ppm range. An example is the 2,4-D kit (Method 4015) developed by Ohmicron which was designed to be sensitive to drinking water MCLs of 18 ppb, and could also be used with appropriate dilution for RCRA toxicity characteristic (TC) analysis at a 10-ppm regulatory limit.

Portability is another advantage of immunoassay technology. Most immunoassay products are sold in kit form and utilize very small apparatus, both for sample handling and in the colorimeter used for detection. Thus, it is very easy to take these kits directly to a field site for use or to an on-site mobile laboratory. However, when working directly on a field site with immunoassay methods, the operator must be very careful to follow the manufacturer's instructions on the package insert as to operating parameters, particularly with respect to effective temperature range. If the method is performed outside of the manufacturer's specified temperature range, i.e. if performed at 10 °C when the specified range is 15 °C to 30 °C, the method will not work and will give erroneous data. Most kits that are operated outside of their designated temperature range are usually designed to give positive results, i.e. potential false positives.

Rapid turnaround time is another advantage of immunoassay technology. Most samples can be run in less than an hour directly on-site. This is particularly useful in a remediation activity where soil is being removed and the site manager needs to know when the cleanup level has been achieved. At this point the excavation can be demonstrated to have been completed. This can result in a considerable cost savings over the conventional approach of sending a few samples out to a laboratory and waiting several days for the analytical results to come back. By this time, a great deal of unnecessary expenditure for excavation or equipment demurrage may have occurred.

Use of immunoassay technology can also improve the overall data quality of a remediation project. The rapid turnaround time and significantly lower cost per sample over conventional laboratory methods, e.g. gas chromatography/mass spectrometry (GC/MS) or gas chromatography/electron capture detection (GC/ECD),

can result in the ability to run a much larger number of samples during the remediation project. The data from these additional samples provide a much better characterization of the site and the progress of the remediation than would the data from the relatively few conventional samples that may normally be analyzed.

The use of immunoassay technology can be very cost effective compared to conventional technologies for all of the reasons previously mentioned in this section. However, since immunoassay methods are used primarily as quantitative screening methods, one still needs to do appropriate confirmation analysis using conventional analytical methods to support the immunoassay data. OSW recommends that approximately 5 to 10% of the immunoassay samples that generate negative results be confirmed by conventional laboratory techniques, depending on the number of total samples that are run for the particular project. Confirmation of positive samples may be done on a routine basis or as needed.

However, despite the many advantages of using immunoassay methods, there are a few disadvantages as well. The primary disadvantage is that, because they are very selective in nature, they are not effective tools for characterization of an unknown site. The most effective use of immunoassay methods is in the monitoring mode for a well-characterized site, rather than in the survey mode when one is trying to determine what compounds are the primary contaminants of the site.

Another disadvantage is that for class-specific methods, the immunoassay kit does not differentiate between the individual analytes present. Also depending on the nature of the antibody employed, the specific method may not be sensitive to all compounds within that particular class. Some of the early PAH kits (Method 4035) who had antibodies designed on phenanthrene were not particularly sensitive to PAHs containing more than four rings. An additional kit sensitive to benzo[*a*]pyrene was needed to analyze for PAHs containing 5 to 7 rings. A second generation kit with an antibody targeted to a four-membered ring (chrysene) now covers the entire range. In addition, some of the early PCB kits (Method 4020) were designed around the congeners present in Aroclor 1248 or Aroclor 1254 and did not respond well to the less chlorinated congeners found in Aroclor 1242. Thus, it is important to have a good site characterization prior to employing immunoassay methods in the monitoring mode.

Another factor of which an operator needs to be aware is the type of potential matrix interferences that may be present on a site to prevent an immunoassay method from performing. Five per cent of an oil could prevent most soil PCB kits from working, except for the one product that was specifically designed to perform in an oily matrix. Moisture levels and pH can also prevent

some kits from performing as expected by the user or claimed by the manufacturer. The precautions necessary to overcome these problems are usually included on the package inserts from the manufacturers.

A peripheral issue is the extent of training necessary for an operator to become proficient in the use of the immunoassay kits. Early on, the sales push was that a minimally trained operator could take a kit, maybe read the instructions, and then go out into the field and start performing analyses. In reality, this was not the case at all. Operators need to be trained well in the nuances of handling immunoassay kits. A non-scientist, in particular, needs to be educated very intensely as to which steps in the procedure are critical and must not be changed. In the validation studies for the first 10 ELISA methods, OSW found that analytical chemists, by far, obtained the best analytical results.

6.2 Implementation Issues

There have been some initial barriers to getting immunoassay methods accepted for routine use in the environmental community. These barriers have been both technical and cultural in nature. The technical barriers include lack of knowledge about analytical options; use of expensive time-consuming methodology when more efficient methodology is available; poor planning of the initial analytical scheme; failure to identify proper questions to be answered which can result in generation of data inappropriate to address the problem at hand. Cultural barriers include inappropriate or excessive regulatory restrictions on use of new methods, e.g. requiring the use of only promulgated methods for program applications that do not have these requirements, and requiring the use of expensive broad-scope methods, e.g. GC/MS, for limited monitoring applications for only a few known and well-characterized analytes.

An additional issue of concern was whether the Regulatory Program Offices could live with analytical values that were not a specified number, i.e. a less than value (usually the regulatory action level) vs. a definite number (0.1 ppm) or a range of values (>5 and <50). We, in the RCRA Program, decided that we could indeed use these values to answer the basic questions for which these analyses were performed, i.e. Have we attained our cleanup criteria? Where do we have to focus our cleanup efforts? We decided that our normal operating procedures for confirming quantitative screening results would be to use the standard reference method to confirm positives and to spot check a certain percentage (usually 10%) of negative results.

Other Program Offices in USEPA, such as the OW may have some restrictions in their current regulations which require them to generate a definite analytical value. The

Office of Groundwater and Drinking Water (OGWDW) has decided to include quantitative immunoassay screening methods in their regulations, the first of which will be Method 4670 for triazines as atrazine. This potential application is described in section 6.5.

OSW has initiated a major effort to educate USEPA permit writers, enforcement people, and others who deal with analytical methods in their jobs as to what the regulatory aspects of using RCRA methods really are. Many of these people were not aware that RCRA regulations did not require the use of only promulgated methods for most applications. The Methods Team has developed a formal training program for RCRA personnel in the Regions and at Headquarters to make them aware of what methods the regulations really allow them to use for RCRA applications, and how to plan an efficient, cost effective sampling and analysis plan.

State programs are a little more difficult. Since RCRA is a Federal Program which has been passed down to most States to administer, the State regulations can be more restrictive and tend to vary greatly. Some States mandate the use of SW-846 methods for all RCRA analytical applications within the State. Flexibility within State Programs varies from allowing only the use of promulgated methods to using any method that may be appropriate for an application. Through dialogue with the USEPA Regions and Headquarters, some of the States are beginning to take an interest in utilizing immunoassay methods. TPHs analysis is the major focus right now in State Programs, since it is not regulated at the Federal level. Several States are beginning to adopt Method 4030 for use in their Underground Storage Tank (UST) Programs, e.g. Georgia and California.

6.3 Examples of Performance Based Measurement Systems

The USEPA has established a policy that, where possible, the Agency will use the PBMSs approach in its monitoring programs for generating environmental data.⁽⁸³⁾ The Agency defines PBMS as a set of processes wherein the data quality needs, mandates or limitations of a program or project are specified, and serve as criteria for selecting appropriate methods to meet those needs in a cost-effective manner.⁽⁹⁴⁾

The PBMS approach has been the basis of the RCRA monitoring program since its inception back in 1980. The inherent flexibility of this approach is an absolute necessity for a program that has responsibility for overseeing analyses in a wide variety of diverse media. The approach is very simple in nature. Rather than focusing on a prescriptive method approach, where following a published method exactly as written is the main requirement, the PBMS approach focuses on a series

of questions to be answered about a specific application. These questions are based on the scientific method and focus on whether a selected method or methods can provide performance appropriate to address the data quality requirements of a particular project. The questions include:

1. What is the purpose of this analysis? (Why are we doing this analysis?)
2. How (for what action) are the data generated from this analysis to be used?
3. What are the data quality needs for this project, i.e. how good does the data have to be for it to be useful for its intended purpose? (Including regulatory drivers, target analytes, matrices, concentration levels, statistical confidence levels, etc.)

Immunoassay methods are the ultimate tools for demonstrating and using this PBMS approach. They are designed and manufactured around specific performance criteria, i.e. methods appropriate for use in Method 4020 are capable of determining whether a soil sample contains PCBs above a concentration of 5 ppm to a 95% upper confidence limit around the mean. There are currently four validated immunoassay products in Method 4020 that can meet these stated manufacturers' performance criteria, all of which utilize different chemistries.

6.4 Applications as Screening Methods

6.4.1 In the Field

OSW decided to take a cautious approach to the introduction of a new technology to the environmental field, with which most analytical practitioners were unfamiliar, and limit the initial applications of immunoassay methods to quantitative screening. We were aware that the technique had been used in Clinical Laboratories for many years in both screening and determinative applications. Since Regulatory Agencies tend to be slow to accept new and different approaches to analysis, anyway, we decided to take a "walk before you run" approach to introducing the new methodology to the people actually doing site assessments and cleanups.

The two primary applications of immunoassay methods in the RCRA Program are mapping of contamination at well-characterized sites slated for cleanup and monitoring the effectiveness of the cleanup activities. Immunoassay lends itself very well to these two particular applications. It is not particularly applicable to the identification and characterization of unknown contaminants at waste sites when compared to much more comprehensive techniques such as GC/MS. However, the reporter gene method (Method 4425) for planar aromatic compounds can be

used to determine the presence of and approximate concentrations of PAHs and chlorinated planar organics and distinguish between them. For monitoring applications of known contaminants, the specificity, sensitivity, and cost effectiveness of quantitative screening immunoassay methods are excellent.

Over the past few years, the general acceptability and willingness to use immunoassay methods within the USEPA Regions for RCRA and Superfund applications has increased exponentially. A significant factor in this change of attitude, in addition to OSW's attempts to educate users in the applicability of the technique, is the specter of shrinking budgets. Field people who are charged with actually doing cleanups are looking for more cost effective ways to do their jobs with less available money. A technique, such as immunoassay methodology, which can generate results of known quality in real-time, and can keep the bulldozers rolling can contribute significantly to reducing the costs of cleanups, and is being looked upon more favorably.

The initial application of immunoassay technology in the RCRA Program was for determining compliance at wood surface treating facilities with PCP regulatory limits. The selectivity and sensitivity of the immunoassay method easily met the regulatory action limit of 0.1 ppm. Use of the PCP immunoassay method (Method 4010) for compliance monitoring was encouraged by OSW and the method was added to SW-846 as a part of the Wood Surface Treatment Rule in 1994.

The major applications for which immunoassay methods are currently used in the RCRA Program are site mapping and monitoring cleanups at sites contaminated with PCBs. Use of the PCB method (Method 4020) has resulted in cost savings at many sites in several Regions. The speed and low cost of the test allows for more extensive mapping of contamination at a site, because many more samples can be analyzed on-site, thus generating a more detailed map of the site. This results in lower cleanup costs, since the cleanup efforts can be directed only at the places that need to be cleaned up, instead of to a broader area. The design of the method allows for rapid determination of whether or not the site cleanup level has been met, thus reducing costs of cleanup in both time and equipment. With the availability of the PAH method (Method 4035) and several types of kits with various sensitivities, the technique is now beginning to be used to monitor sites contaminated with PAHs.

Another major application within OSWER is for mapping and cleanup of sites contaminated with petroleum hydrocarbons from leaking storage tanks partially for the Office of Underground Storage Tanks (OUST) and primarily for the States. The TPH method (Method 4030) is effective for determining gasoline, diesel, kerosene, and jet fuel at most State-required cleanup levels.

The recent Department of Defense cutbacks set into motion a large number of military base closures in the United States, resulting in a plethora of site cleanup projects. The primary analytes of concern for these base closure cleanups were explosives, petroleum hydrocarbons and PCBs, all of which could be monitored using immunoassays and other screening techniques. The immunoassay ELISA methods for TNT (Method 4050) and RDX (Method 4051) have been used extensively along with the immunosensor methods (Methods 4655 and 4656) and the colorimetric screening Methods 8010 (for RDX) and 8015 (for TNT).

The ELISA method for mercury (Method 4500) has been used effectively to monitor the progress of the soil cleanups of several Superfund sites contaminated with mercury.

6.4.2 *In the Laboratory*

Immunoassay methods have been used in many laboratories to screen samples for high levels of contaminants to prevent instrument downtime. The dioxin screening method (Method 4025) was primarily designed for use in a laboratory, rather than in the field, because of the detailed sample preparation procedures involved. An effective dioxin screening method can result in significant cost savings for monitoring dioxin-contaminated sites. A negative immunoassay screening result can eliminate the need for a high resolution GC/MS analysis costing between \$1500 and \$2000 each. The method has been validated on a set of 56 real world soil samples and gave results that were 91% correct with 9% false positives. No false negatives were reported. It has also been used to screen river sediment samples and food samples potentially contaminated with dioxin.

6.5 Applications as Quantitative Methods

At the time of writing, there are very few quantitative applications for immunoassay methods. There are a few being developed. USEPA's Drinking Water Program is planning to incorporate Method 4670 for triazines as atrazine into its screening regulations. Current regulations require that drinking water suppliers test their supplies quarterly for atrazine at an MCL of 3 ppb. Method 4670 is appropriate for this measurement requirement, because it can measure these compounds sufficiently below the 3 ppb MCL to be effective. Since it also has a positive crossreactivity to other triazines, e.g. simazine and the like, the data generated as atrazine would be an overestimation and thus would be a conservative measurement.

Some of the more selective screening methods, e.g. TNT, RDX, and 2,4-D were originally submitted as screening methods for reasons described previously.

However, they are sufficiently selective to allow their use as quantitative methods. The TNT methods have been used for some quantitative applications on military base cleanups. They tend to generate high results with respect to TNT, because they also exhibit significant (and desirable) crossreactivity to TNT breakdown products such as DNT and DNB.

7 FUTURE DIRECTIONS

The environmental immunoassay method program has come a very long way, from nonexistent in early 1992 to a significant and viable program with a variety of available methods utilizing several different immunoassay technologies today. However, we still intend to continue to push ahead and keep the new methods coming and remain current with the state-of-the-art.

The primary focus right now is on completing the validations of the dioxins and coplanar PCB methods in Table 3, which is expected to be done by early 2000. We are also interested in continuing with the development of additional quantitative immunoassay methods for specific target analytes. We are continuing to investigate the applicability of immunoassay in the area of affinity chromatography.

Another area of interest for continued development is that of immunosensors. Several Federal Agencies are interested in further developing this technology beyond the realm of explosives. We are in the process of planning some preliminary projects, beginning in 2000, to complete the development of new immunosensors for PAHs, PCBs, and TCE.

Now that the industry has sorted itself out after the mergers of 1997, there are a number of small, energetic companies with a broad base of expertise in a wide variety of immunoassay techniques. We, in OSW, plan on continuing our productive relationship with these industrial partners to bring the best of immunoassay technology to bear on future environmental problems.

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methods with whom I have worked over the years, particularly Steve Friedman of Sylvanus Laboratories, who has been my primary mentor in immunoassay technology over the years.

ABBREVIATIONS AND ACRONYMS

APAH	Anthracene Polynuclear Aromatic Hydrocarbons
B[a]PEqs	Benzo[a]pyrene Equivalents
CAA	Clean Air Act
CBI	Confidential Business Information
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CPAH	Carcinogenic Polynuclear Aromatic Hydrocarbons
CWA	Clean Water Act
DELFLIA	Dissociation-enhancement Lanthanide Fluoroimmunoassay
ELISA	Enzyme-linked Immunosorbent Assay
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GC/ECD	Gas Chromatography/Electron Capture Detection
GC/MS	Gas Chromatography/Mass Spectrometry
HPLC	High-performance Liquid Chromatography
MCL	Maximum Contaminant Level
OAR	Office of Air and Radiation
OARM	Office of Administration and Resource Management
OERR	Office of Emergency Response and Remediation
OGWDW	Office of Groundwater and Drinking Water
OPPTS	Office of Prevention, Pesticides and Toxic Substances
ORD	Office of Research and Development
OSW	Office of Solid Waste
OSWER	Office of Solid Waste and Emergency Response
OUST	Office of Underground Storage Tanks
OW	Office of Water
PAH	Polynuclear Aromatic Hydrocarbon
PBMS	Performance Based Measurement System
PCB	Polychlorinated Biphenyl
PCDDs	Polychlorinated Dibenzo- <i>p</i> -dioxins
PCDFs	Polychlorinated Dibenzofurans
PCP	Pentachlorophenol
RCRA	Resource Conservation and Recovery Act

RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
RLUs	Relative Light Units
SCHC	Short-chain Hydrocarbons
SDWA	Safe Drinking Water Act
TC	Toxicity Characteristic
TCDD	2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin
TCE	Trichloroethylene
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TNT	2,4,6-Trinitrotoluene
TPH	Total Petroleum Hydrocarbon
TSCA	Toxic Substances Control Act
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
UST	Underground Storage Tank
2,4-D	2,4-Dichlorophenoxyacetic Acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic Acid

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General Articles (Volume 15)
Analytical Problem Solving: Selection of Analytical Methods • Quality Assurance in Analytical Chemistry

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