IMMUNOASSAY METHODS: DEVELOPMENT AND IMPLEMENTATION PROGRAM AT THE USEPA

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Introduction

The USEPA has been looking at the potential use of immunoassay technology for environmental monitoring for several years. 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.

Validation Criteria for Immunoassay Methods

In addition to the guidelines for developing screening methods in general, OSW, based on its own experience and that of other regulatory Agencies (FDA), has generated some validation criteria specifically applicable to immunoassay methods. These validation criteria are described in a guidance document. Data generated to meet these criteria 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, and
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-dichlorophenoxyacetic acid (2,4-D) as well as the 2,4-D, and for other 3-, 4-, and 5-ring polynuclear aromatic hydrocarbons (PAH) when testing for phenanthrene in a PAH screening method.

The false negative/false positive rate for a particular immunoassay kit is very important. Definition of a false negative is a negative response for a sample that contains the target analyte(s) at or above the stated action level of the method. Definition of a false positive is a positive response for a sample that contains analytes at less than the action level. 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 is 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.

The performance data generated from environmental samples spiked with the target analytes gives 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. Manufacturing quality control and validation information gives a good indication as to continued test kit availability.

Up to this time, all of the immunoassay test kits (10-15) that the OSW has evaluated have been extensively tested and validated by the manufacturers. EPA 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 EPA Regional laboratories.

Status of the EPA Immunoassay Methods Development Program

Several EPA Program Offices are investigating the potential applicability of immunoassay methods to their programs. However, the OSW is the first EPA Program Office to formally incorporate these methods into its methods program. As of June, 1995, OSW has completed validation of ten immunoassay methods utilizing approximately 15 kits, and is in the final stages of validating several new methods and additional kits for existing methods. The validated methods are Method 4010-Pentachlorophenol (PCP) in Water and Soils by Immunoassay, Method 4015-2,4-D in Water and Soils by Immunoassay, Method 4020-Polychlorinated Biphenyls (PCBs) in Soil and Oil by Immunoassay, Method 4030-Total Petroleum Hydrocarbons (TPH) in Soil by Immunoassay, Method 4035-Soil Screening for Polynuclear Aromatic Hydrocarbons (PAHs) by Immunoassay, Method 4040-Toxaphene in Soils by Immunoassay, Method 4041-Chlordane in Soils by Immunoassay, Method 4042-DDT in Soils by Immunoassay, Method 4050-TNT Explosives in Water and Soils by Immunoassay, and Method 4051-RDX Explosives in Water and Soils by Immunoassay.

Potential Environmental Applications for Immunoassay Methods

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 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 gas chromatography/mass spectrometry (GC/MS). However, for monitoring applications of known contaminants, its specificity, sensitivity, and cost effectiveness are excellent.

Over the past two years, the general acceptability and willingness to use immunoassay methods within the EPA 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 high-quality results 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 approved for inclusion in SW-846 as a part of the Wood Surface Treatment Rule.

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 recent availability of the PAH method (Method 4035), the technique is now beginning to be used on sites contaminated with PAHs.

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

Additional analytes have been targeted, including pesticides, herbicides and explosives, as new methods are developed and validated. Eventually, OSW intends to perform quantitative analysis either using immunoassay methods for direct quantitation or as concentration techniques using affinity chromatography, with quantitation by existing techniques, e.g. HPLC or GC/MS. The latter approach will be particularly effective for doing analyses where multiple analytes within a class need to be individually identified.

Barriers to Use of Immunoassay Methods

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 resulting 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 EPA, such as the Office of Water may have some restrictions in their current regulations which require them to generate a definite analytical value. If this is indeed the case, their focus would be on quantitative immunoassay methods rather than screening methods.

OSW has initiated a major effort to train EPA permit writers, enforcement people, and others who deal with analytical methods in their jobs in the regulatory aspects of using RCRA methods. Historically there have been problems where only promulgated methods were allowed to be used in many applications under the RCRA regulations where this was not a requirement. The Methods Section has developed a formal training program for RCRA personnel in the Regions and at Headquarters to make them aware as to which methods are allowable and appropriate to use under the RCRA regulations in both mandatory and non-mandatory applications, and how to prepare efficient, cost effective sampling and analysis plans.

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 EPA Regions and Headquarters, some of the States are beginning to take an interest in utilizing immunoassay methods. TPH 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.

Summary and Conclusions

The OSW immunoassay methods program was initiated in January, 1992, with the evaluation of the screening method for PCP. Methods for PCBs and TPH followed soon after. EPA has proposed a total of ten screening methods for inclusion in Update 3 of SW-846. For a Regulatory Program, immunoassay methodology has advanced very rapidly.

There was initially a general reluctance among the regulatory and regulated community to use immunoassay methods, even for applications for which they were appropriate. This was due to a lack of knowledge about the technology and a belief among both the regulators and regulated community that only promulgated SW-846 methods could be used for all RCRA applications.

The climate has changed considerably during the past two years regarding the use of screening methods in general, and immunoassay methods in particular in the environmental community. We have noticed a much greater willingness for EPA Regional and some State regulators to allow for the use of immunoassay methods in their RCRA Programs. Apparently, the dissemination of information about the effective performance of immunoassay methods and a budget crunch which drives both regulatory and remediation personnel to look for more cost effective means to do their jobs have begun to have an impact in the environmental community.

The future looks bright for the environmental application of immunoassay methodology. Many other Federal Agencies with massive cleanup problems, e.g. The Department of Energy (DOE) and The Department of Defense (DOD), have become interested in the technology for its overall utility in significantly cutting the costs of cleanup operations. Within the next year, we expect to focus more on quantitative immunoassay methods. We believe that the environmental community has reached an appropriate comfort level with using immunoassay screening methods, and we will begin to introduce the quantitative methods. The EPA will continue its cooperative effort with the immunoassay manufacturers and other methods developers to develop the methods that are needed for its environmental programs.