Environmental Immunochemical Analysis for Detection of Pesticides and Other Chemicals

A User's Guide

Shirley J. Gee Bruce D. Hammock
Jeanette M. Van Emon

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A User's Guide

by

Shirley J. Gee **Bruce D. Hammock**

Department of Entomology
University of California
Davis, California

Jeanette M. Van Emon

Environmental Monitoring Systems Laboratory
Las Vegas, Nevada

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EXECUTIVE SUMMARY

Immunochemistry has broad applications for a wide variety of environmental contaminants. However, the potential for applying immunochemical methods to environmental measurements is just beginning to be realized. Immunochemical methods are based on specific antibodies combining with their target analyte(s). Many specific antibodies have been produced for targets of environmental and human health concern. Such antibodies can be configured into various analytical methods. The most popular immunochemical technique in environmental analyses today is immunoassay. Immunoassays have been shown to detect and quantify many compounds of environmental interest such as pesticides, industrial chemicals, and products of xenobiotic metabolism. Among the most important advantages of immunoassays are their speed, sensitivity, selectivity and cost-effectiveness.

Immunoassays can be designed as rapid field-portable, semi-quantitative methods or as standard quantitative laboratory procedures. They are well suited for the analysis of large numbers of samples and often obviate lengthy sample preparations. Immunoassays can be used as screening methods to identify samples needing further analysis by classical analytical methods. Immunoassays are especially applicable in situations where analysis by conventional methods is either not possible or is prohibitively expensive.

Environmental immunoassays have broad applications for monitoring studies. The EPA has used immunoassay methods for monitoring groundwater and cleanup activities at hazardous waste sites. Immunoassays can also be used as field screening tools to confirm the absence and or presence of particular contaminants or classes of contaminants for special surveys. Other federal and state agencies are employing immunoassay technology where appropriate such as for extensive monitoring studies that generate a large sample load.

In addition to detection methods, other immunochemical procedures can be used for environmental analysis. Immunoaffinity techniques now used extensively in pharmaceutical and biotechnology applications can be adapted to extract, and cleanup environmental samples. Selective and sensitive sample collection systems such as air and personal exposure monitors can be designed based on the principal of immunoaffinity. Although immunoaffinity procedures are not addressed in this tutorial, they are mentioned here to illustrate to the reader that immunochemical methods can be adapted to a wide variety of monitoring situations.

The U.S. EPA Environmental Monitoring Systems Laboratory at Las Vegas, Nevada (EMSL-LV) has a program to develop and evaluate immunochemical methods for environmental analysis. The EMSL-LV immunochemistry program consists of the following major components: identification of need for an immunochemical method, identification of existing technologies, development of new technologies, adaptations of existing technologies, evaluations of existing technologies, field demonstration of portable technologies, and finally technology transfer. Overall program goals, as well as prioritization of compounds for methods development, are based upon input from client EPA Program Offices as well as the EPA Regions. Analytical needs are defined as to target analytes, matrices, detection limits and application of the method.

Methods and immunologic reagents have been developed for the polychlorinated biphenyls (PCBs), BTX (benzene, toluene, xylene) and various pesticides and nitroaromatic

compounds through the EMSL-LV immunochemistry program. Additional methods are under development for pyrethroid and organophosphorus pesticides.

The EMSL-LV immunochemistry program conducts laboratory and field evaluations to assess method performance. The evaluation, characterization and testing of a particular analytical method is necessary to ensure the intended use of the method is met. Evaluations are conducted according to EPA guidelines requiring the determination of precision, within and among laboratories bias, method detection limit, matrix effects, interferences, limit of reliable measurement and ruggedness of the method. Demonstrations under the Superfund Innovative Technology Evaluation (SITE) program have been used to document method performance under real-world environmental conditions. SITE demonstrations of immunoassay methods for the PCBs, pentachlorophenol, and BTX have been completed, other demonstrations are being planned. After a SITE demonstration the methods can be submitted to the Superfund Field Screening Methods compendium for inclusion and distribution.

Technology transfer activities include providing guidance and training to EPA regional, EPA headquarters, and state personnel on the use of immunoassays. A computer animated graphics program has been developed to provide instruction on the theory and applications of immunoassays. This graphics program may be a useful training aid to the tutorials contained in this document. Other instructional activities planned include the development of training videos for performing immunoassays. A "hands on" workshop at the EMSL-LV is also being considered. Individual training for EPA personnel has been conducted and will remain an option for interested individuals.

Another vehicle to facilitate the implementation of immunochemical methods are annual meetings of researchers, developers and end-users of immunochemical methods. The EMSL-LV has sponsored two meetings to discuss the direction of immunochemical methods research, development, application, and acceptance within the regulating and regulated communities. The last Immunochemistry Summit Meeting was held in September 1993 and included representatives from EPA and other federal and state agencies, large chemical companies, biotechnology companies, and research institutes. It is anticipated that this type of meeting will continue to be an annual forum for concerns and issues regarding environmental immunochemical methods.

Considering the advantages and versatility of immunochemical methods, it is surprising that the technology has not been more widely accepted by environmental analytical chemists. Although many immunoassay methods have been reported in the literature, their potential has not been practically realized. Part of the problem is misunderstanding and perhaps skepticism on the part of analytical chemists. A thorough understanding of the advantages and limitations of immunoassay methods is essential to applying the technology in situations where they offer the most promise. It is the intent of this document to dispel the mystery in understanding and performing an environmental immunoassay.

This document presents six specific immunoassay methods. The methods are based on the same working principle but illustrate different applications of the technology for various analytical situations. Two methods are presented to describe immunoassays for lipophilic analytes using the triazine herbicides as examples. Although either method can be used for environmental samples, both are presented to illustrate different formats for the same analyte. The third method is for the insecticide carbaryl which is applicable for both environmental and

biological samples. Methods for p-nitrophenol, paraquat and for various triazine mercapturates are examples of water soluble analytes. The triazine mercapturates method illustrates the application of immunoassay for urine samples and hence exposure assessment studies. Accompanying solid phase extraction procedures to extract triazines from water and atrazine mercapturate from urine are also provided. All of the methods described are intended to serve as examples of the utility of immunoassay technology.

In addition to the six specific immunoassay methods, this document also describes analytical laboratory techniques necessary to perform immunoassays. Suggestions for general laboratory considerations such as protocol design, sample preparation, data handling and analysis, and safety precautions are also given. Examples of troubleshooting and quality control practices are included which can be applied to assays not contained in this tutorial. Protocols for preparing buffers, determining reagent integrity and for optimizing assay conditions are also useful for immunoassays in general. An appendix of commonly used terms in immunoassay should facilitate understanding of the technology.

Although immunoassays are now being employed for environmental analysis, there may still be a need for training non-analysts in the use of immunoassay or updating the experienced analytical chemist on an unfamiliar analytical format. It is hoped that the methods and procedures found in this users guide will be beneficial and help to standardize the immunochemical analysis of small molecules. Comments and written requests for additional information may be directed to Jeanette M. Van Emon, U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, P. O. Box 93478, Las Vegas, Nevada 89193-3478.

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PREFACE

Immunochemical methods for the analysis of environmental contaminants are relatively new on the analytical chemistry scene. These methods are based on the use of a specific antibody as a detector for the analyte of interest. Immunoassays are rapid, sensitive, and selective, and are generally cost effective for large sample loads. They have been applied to diverse chemical structures (i.e. triazines, sulfonylureas, organophosphates, polychlorinated biphenyls, cyclodienes) and are adaptable to field use. These characteristics make immunochemical analysis a valuable tool for use by the environmental analytical chemist. This document is designed to facilitate the transfer of this valuable technology to the environmental analytical chemistry laboratory. Field personnel who may need to employ a measurement technology at a monitoring site may also find this manual helpful.

This document is a tutorial designed to instruct the reader in the use and application of immunochemical methods of analysis for environmental contaminants. A brief introduction describes basic principles and the advantages and disadvantages of the technology, and gives a listing of references which supply more detail. Preparation of the laboratory for use of this technology and the general scientific considerations prior to using the technology are discussed. Detailed step-wise procedures are given for analysis of selected analytes, triazine herbicides, carbaryl, paraquat, and p-nitrophenols in environmental samples as well as triazine mercapturates in urine samples. In addition to the specific immunoassay methods, a series of support techniques necessary to perform immunochemical methods are described. These support techniques include pipetting, sample preparations, testing for matrix effects, optimizing reagent concentrations, data analysis, recordkeeping, and equipment maintenance. A general troubleshooting guide is provided to aid both the novice and experienced analyst.

This document provides specific instruction for certain analytes, but also serves to familiarize the novice reader with many generic concepts needed to successfully utilize immunochemistry technology including: applications, sampling, sample preparation, extraction, cleanup, quality assurance, methods development and optimization, data handling and troubleshooting. It is not necessary for the reader to actually perform the immunoassays given in this User's Guide to obtain familiarity with these concepts. The Guide is written so that the information presented can be applied to other immunoassays not given here. Thus, the strength of the Guide is its universal applicability to immunoassay methods.

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SECTION 1
INTRODUCTION

This manual is designed to introduce the analytical chemist to the general concepts and use of immunoassays for the analysis of pesticides and other small molecules. By writing this manual we hope to encourage the analytical chemist to consider this technology among the repertoire of methods available for solving analytical problems. As with any other analytical technique, it will be just as important for the analyst to be able to identify when immunochemical technology is appropriate, as it is to learn how to conduct an immunochemical analysis. Field personnel who may need to employ a measurement technology in the field may also find this manual helpful.

The manual is organized first to provide some general information on the technology, second to provide tutorials consisting of some specific examples of immunoassays and thirdly to provide guidelines and information on those procedures specific to immunochemical techniques which may not currently be in use in the typical analytical chemistry laboratory. All of these procedures were developed in an academic laboratory and may need to be adjusted to meet regulatory requirements of the various agencies within the government as to method performance and their procedural guidelines. This caveat also can apply to a Contract Laboratory.

1.1 Brief history

The development of chromatographic instrumentation by pesticide analytical chemists was closely paralleled by the development of immunoassay techniques by clinical analytical chemists. Immunoassays are routinely used in clinical situations for the analysis of proteins, hormones and drugs. The success that these immunochemical procedures has achieved in the clinical area is being transferred to the area of pesticide analysis.

The first antibodies developed against pesticides were reported in the 1970's. Recently this technology has been refined for use in the pesticide analytical chemist's laboratory to the point that commercial test kits are now available. With the availability of kits, it is imperative that the analyst understand the underlying principles of the methodology in order to evaluate the strengths and limitations of any one "kit" for their specific application. Since many of these easy-to-use test kits are designed for the non-analyst, it is important that these users also have the same fundamental understanding.

1.2 Advantages/Disadvantages

Immunoassays are a useful complement to the analytical chemist's repertoire of methods for the detection of pesticides and other environmental chemicals. Immunoassays are rapid, sensitive and selective for the analyte of interest and generally cost effective for large sample loads. Immunoassays have been applied to diverse chemical structures and are adaptable to field use. As with any technology there are disadvantages. Antibodies may bind to structural analogs of the analyte of interest (termed cross-reactivity). This technology is not easily adapted to a multianalyte method, since each antibody binds primarily to a single analyte or class of analytes. Reagent stability is often cited as a problem, but can be overcome based on knowledge gained from the clinical field. This technology also requires a large sample load to justify development of a new assay for an analyte of interest, due to the expense of producing antibodies and establishing the procedure. For intermittent analysis, it might be more cost effective to use existing commercially prepared test kits.