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OFFICE OF RESEARCH AND DEVELOPMENT

TECHNOLOGY SUPPORT PROJECT

EPA

Immunochemical Analysis of Environmental Samples

U.S. EPA Human Exposure Research Branch
Immunochemistry Program

The Need

Field methods used for detecting compounds of environmental significance traditionally have been derived from standard laboratory methods. When laboratory methods are adapted to the field, they are often relatively slow, insensitive, expensive, and require bulky transportable equipment and skilled operators. There is a need for rapid, sensitive, low-cost, portable, and simple field methods for analysis of environmental samples. Immunochemistry offers those advantages. The only specialized equipment needed is a spectrophotometer, microtiter plates or test tubes, precision pipets, and immunologic reagents.

Commercial manufacturers sell kits for field screening, and new equipment and methods are being developed for rapid, accurate field analysis of a wide variety of analytes, such as heavy metals, dioxins, and PCBs, that are found at Superfund and RCRA sites. As a result the regulator and regulated communities view immunochemistry as a powerful technology for screening analysis of environmental contaminants.

Immunochemistry includes techniques such as immunoaffinity chromatography and immunoassay. Sample preparations based on immunoaffinity take advantage of the attraction between an antibody and a specific analyte. The procedure has great potential for cleanup of complex samples like soils and sludges. By rinsing a sample over an antibody-treated surface, chemists can isolate particular compounds that adhere to the antibody. The isolated compound is then eluted from the immobilized antibody and is ready for analysis by chromatography or immunoassay. One common immunoassay is the enzyme-linked immunosorbent assay (ELISA). In this technique, the selectivity of the antibody for the analyte and the resultant antibody-analyte complex is the basis for the specificity of immunoassays.

The Use

The Human Exposure Research Branch in Las Vegas (HERB), part of the National Exposure Research Laboratory, is pioneering an investigation into the usefulness of immunochemical techniques for monitoring the extent of contamination in environmental and biological matrices. HERB has developed and demonstrated several of these techniques and believes that they hold great promise for the quantitative analysis of target analytes for use in groundwater surveillance, *in situ* hazardous waste site monitoring, and assessment of human exposure. Current work involves the analysis of chemicals like PCBs, nitroaromatics, and certain pesticides that are difficult to analyze by other analytical methods. HERB has sponsored two national meetings that focused on regulatory issues and technological advances in environmental immunochemistry. These meetings brought together government, industry, and university scientists to discuss problems of mutual interest in the field.

A 1993 Technology Support Center project at a Superfund site in Region 5 demonstrated the usefulness of immunochemical methods for screening PCBs in soil and river sediment. This project was an example of cooperation between EPA, DOE, the state of Michigan, and various contractors. Two immunoassays and a chlorideion specific electrode were used on site and the real-time analytical results were compared with standard GC results from EPA method 8081. Preliminary results show good agreement between the immunoassays and GC and even stronger correlation could be achieved with tighter quality control measures.

In addition, other EPA offices have applied immunochemistry for screening and analysis in their programs. The Office of Water has used immunoassays to screen indirect discharges of specific analytes for permitting under the Clean Water Act (304h). Sample analysis data may soon be used for comparison and compliance monitoring within selected industries, such as commercial laundries. The Office of Pesticides is looking at ways to shorten the pesticide registration process by using immunochemistry as a cost-effective technology.

Other government agencies and universities are studying immunochemical methods. The Food and Drug Administration (FDA) may use immunoassays to obtain data for the calculation of safe concentrations of residues. A recent university project used immunoassays to track contamination during the 1993 Midwestern flood. In applications as diverse as organic geochemistry and military

The Use
Continued

operations, immunochemical methods have been used for volatile organic compound measurement. The U.S. Department of Agriculture (USDA) is integrating immunoassays into rapid test procedures for detection of residues in meat and poultry. Results from these tests will be used in regulatory and compliance programs for veterinary drugs, sanitation, and pest control. The National Institute for Occupational Safety and Health (NIOSH) has applied immunoassays to herbicide research, clinical analysis, biomarkers, and immune biomonitoring. They use the methods to detect morphine factor, alachlor, atrazine, cyanazine, metalachlor, and 2,4-D. State laboratories have analyzed soil samples and water from private wells using immunochemical test systems for triazine (atrazine) samples.
The results of EPA's Superfund Innovative Technology Evaluation (SITE) studies indicate a strong correlation between field immunoassays, laboratory immunoassays, and gas chromatography-mass spectrometry.

Another field use of immunochemistry that is being explored at HERB, the personal exposure monitor (PEM), may revolutionize safety and exposure requirements for workers who deal with hazardous chemicals. Immunochemical dosimeter badges can be used to detect pentachlorophenol and nitroaromatics, and are being developed for parathion and chloropyrifos. These badges are lightweight, inexpensive, quick, and provide a real time indication of exposure.

The Limits

The use of immunochemical techniques is gaining acceptance in the environmental sciences. One need that is being addressed is that of specificity. Frequently, immunoassays are available for a class of compounds, like PCBs. Specific quantitation for each component has been difficult.

The development of PEMs, for example, must address the question of diffusion of chemicals through a semipermeable membrane, the optimum concentration of the antibody, detection limits of the PEM and quantitation by immunoassay, the efficiency of the antibody in capturing the analyte, and the capacity of the device.

Validation studies of reproducibility, matrix effects, field trials, false negatives/positives, and correlation with other tests will assist acceptance of immunochemical methods at Superfund and RCRA sites. The legal defensibility of immunochemical results is yet to be determined.

Advantages and limitations are summarized below.

Advantages	Limitations
• Field portable • User friendly • Quick and inexpensive • Potential for wide range of analytes • Useful for many matrices • Low detection limits	• Separate immunoassay needed for each analyte • More complex analysis required for quantitation of specific analytes • Long development time for new antibodies and methods

The Status

One new avenue of investigation is the use of antibody-coated, fiber-optic immunosensors. Another application is the integration of robotics capability for high sample throughput and the development of a tiered analytical approach, i.e., biological and environmental samples, biomar-kers, target analytes, and degradation products. This system of analytical procedures will enable scientists to measure contamination at the source, follow the fate and transport of residual amounts, and assess human exposure. Multi-analyte immunoassays that can identify several analytes are expected to expand the desirability of immunoassay technology for environmental use. Work in this area is already underway at HERB and elsewhere. Other applications of immunochemistry, such as multianalyte optical immunobiosensors and biorefractometry, are being developed.
Industry recently formed the Analytical Environmental Immunochemistry Consortium (AEIC), which is focussing on performance-based method guidelines, method validation, and formation of consensus on regulatory and technological issues. The National Technology Transfer Center (NTTC) offers a vehicle for collaborative studies. Cooperative Research and Development Agreements (CRADAs) between industry and the government can be used to promote technology development and licensing of immunochemical applications. The HERB has a Technology Transfer Office that is able to coordinate CRADAs for the development of immunochemical methods.

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