Immunochromatographic applications in Environmental Science

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Immunochromatographic methods are based on selective antibodies combined with a particular target analyte or analyte group. The specific binding between antibody and analyte can be used to detect environmental contaminants in a variety of sample matrices. Immunoassay methods provide cost-effective, sensitive, and selective analyses for many compounds of environmental and human health concern. Immunoaffinity chromatography methods have been integrated with chromatographic methods and are also being used as efficient sample preparations prior to immunochemical or instrumental detection. Immunosensors show promise in obtaining rapid online analyses. These and other advancements in immunochromatographic methods continue the expansion of their role from field screening methods to highly quantitative procedures that can be easily integrated into the environmental analytical laboratory.

Immunochromatographic methods were first applied in clinical situations where their sensitivity and selectivity were used for diagnostic purposes. By leveraging the sensitivity and flexibility of the interaction between an antibody and antigen, clinical chemists were able to develop highly successful diagnostic methods for medical research and health-care applications. In the 1970s, pesticide chemists realized the potential benefits of immunochromatographic methods, particularly immunoassays (1). Following this transition of the technology, government regulatory agencies became interested in innovative methods for analyzing soil, water, food, and other matrixes of environmental and human exposure significance.

Commercial vendors, responding to the need for simple and matrixes of environmental and human exposure significance. Table 1 illustrates the conceptual similarity between immunoassay and chromatographic methods development (7). Immunoassays can provide rapid screening information or quantitative data to fulfill rigorous data quality objectives. Quantitative immunoassays have been a research focus of NERL-LV since the late 1980s. These
methods have been used increasingly for the analysis of pesticides in food, water, and other matrices (8, 9).

Immunoassays, because of their sensitivity, selectivity, and tendency toward a positive bias, have proven reliable for measurement of various contaminants at trace concentrations. Immunoassays can also provide supplemental data by detecting complex environmental or biological conjugates (products of environmental weathering or metabolism) not amenable to instrumental methods. Immunoassays are also available for several industrial chemicals, including polychlorinated biphenyls (PCBs), pentachlorophenol (PCP), and trinitrotoluene (TNT; 10). A more recent application of immunochemical methods is in the area of human exposure assessment. Environmental contaminants may pose a threat to humans via ingestion, inhalation, or dermal absorption pathways. These routes of exposure often pose analytical challenges. Human exposure assessment studies usually require the analysis of large numbers of samples (e.g., routine urinary screening of workers in a chemical manufacturing plant, or monitoring large subpopulations to determine background levels of xenobiotic metabolites). High sample throughput is a feature of immunoassays that makes them particularly suited to field studies and large-scale monitoring efforts. In the late 1980s, the EPA Superfund Program saw the advantage of immunoassays for site screening and evaluated several methods through the Superfund Innovative Technology Evaluation (SITE) program (11). The SITE program provided some of the earliest field data for evaluating immunoassays.

**Development Steps**

Compounds of environmental concern are usually of low molecular weight. Antibodies for such small molecules can be difficult to develop because, although they may be antigenic, they cannot stimulate antibody production. The small molecule, or frequently a derivative of the compound (termed a hapten), must be conjugated to a carrier molecule, such as a protein or polymer to form an immunogen. The hapten portion of the immunogen should mimic as closely as possible the structure of the target molecule: its size, shape, and electronic properties. Frequently, when forming the hapten, a chemical functionality such as OH, COOH, NH$_2$, or SH is introduced onto the target analyte for conjugation with a carrier protein. Distancing the small hapten from the large carrier molecule with a tether or handle provides a better target for the immune response. The placement of the tether or handle usually influences the selectivity of the desired antibodies. Hapten synthesis should take into account the location of the handle on the target molecule, the length and inertness of the handle, the functional group for coupling, and the solubility and stability of the hapten and conjugates (12). The ideal approach is to develop a large library of haptens for antibody production and methods development. It is important to remember that antigenic analytes can combine with antibodies in the absence of the protein carriers, provided that antibodies of appropriate selectivity have been developed.

A common practice in determining the composition of hapten–protein conjugates is to use UV spectroscopy or titration of unsubstituted amino groups. However, when combining high performance liquid chromatography (HPLC) with UV detection, it is possible to obtain detailed information about conjugate composition, the type and number of substituted amino groups, and their position.

Immunoassay performance is a function of the affinity and selectivity of an antibody for an antigen that results in a product (antibody–antigen) that can be measured (13). Immunoassays are physical assays because the formation of an antibody–antigen complex is characterized by the Law of Mass Action. The most common antibody type used in immunoassays is the divalent immunoglobulin G (IgG). IgG consists of 4 polypeptide chains, 2 identical heavy chains, and 2 light-chains. In the case of IgG, 2 of these monomers associate through the formation of disulfide bonds and form the intact IgG molecule.

There are several types of enzyme immunoassays (EIA), the 2 broadest being heterogeneous (wherein a separation of

<table>
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<tr>
<th>Table 1. Conceptual similarity between GC and immunochemical methods development (adapted from reference 7, and reprinted with permission)</th>
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<tr>
<td><strong>Gas chromatography</strong></td>
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<td>Column coating</td>
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<td>Column length</td>
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<td>Number of theoretical plates</td>
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<td>Gases</td>
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<td>Detector (FID, ECD, NP, TCD, PID, Hall)</td>
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<td>Samples processed serially</td>
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<td>Electronic signal</td>
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<td>Quantitation by peak height, area</td>
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Table 1. Conceptual similarity between GC and immunochemical methods development (adapted from reference 7, and reprinted with permission)
bound and free antibody is required) and homogeneous (requiring no such separation). Homogeneous immunoassays are highly matrix dependent because the color or turbidity of samples interferes with the signal from the colored end-product of the assay. Environmental samples are typically colored, therefore, heterogeneous assays are usually necessary for these applications. There are examples of successfully using a heterogeneous assay when a homogeneous assay has failed (14).

An immunoassay may be based on direct binding or indirect competition. Several immunoassay protocols are available either for specific or widespread applications (8). For example, antibody and analyte may be added directly to the antigen-coated wells of a microtiter plate or, in the case of a direct competition assay, the antibody may be immobilized on a solid phase and the analyte in the sample competes with a known amount of labeled analyte for binding sites on the antibody. Indirect competitive enzyme-linked immunosorbent assays (ELISAs) are commonly used for small molecules, such as pesticides. The methods most frequently used in the environmental analytical laboratory are based on the 96-well microtiter plate format. The microtiter plate enables the simultaneous analysis of a large number of samples, standards, and several quality assurance (QA) performance checks. Other solid surfaces such as test tubes, filter papers, membranes, and even capillary tubes have been employed. Many types of labels can be used for visualizing the reaction between an antibody and antigen, including: enzymes, radioactivity, fluorescence, phosphorescence, chemiluminescence, and bioluminescence. Enzymes with colorimetric substrates are the most common labels in environmental applications with quantitation based on color intensity, rate of reaction, or electrochemical measurements.

Most of the immunoassays presented here are based on indirect inhibition. In this type of ELISA, a constant amount of anti-analyte antibody is incubated with increasing amounts of analyte in individual test tubes. This incubation period allows analyte–antibody complexes to form in solution. The number of analyte–antibody complexes formed and the quantity of remaining free reactants are dependent on the amount of analyte present in samples or standards. The next step is the addition of this incubation mixture to prepared wells of microtiter plates. There, the immobilized coating antigen competes with remaining free analyte in solution for unoccupied antibody binding sites. A washing step removes all material not bound to the microtiter well. A second antibody that is covalently bound and free antibody is required) and homogeneous (requiring no such separation). Homogeneous immunoassays are highly matrix dependent because the color or turbidity of samples interferes with the signal from the colored end-product of the assay. Environmental samples are typically colored, therefore, heterogeneous assays are usually necessary for these applications. There are examples of successfully using a heterogeneous assay when a homogeneous assay has failed (14).

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**Monoclonal and Polyclonal Antibodies**

Monoclonal antibodies are produced by fusing antibody-producing spleen cells with mutant tumor cells (15). These tumor cells are derived from myelomas—cancerous murine plasma cells. Somatic cell hybridization enables the fusion of a myeloma cell with an antibody-producing spleen cell from an immunized animal. The hybrid cells, or hybridomas, exhibit characteristics of each parental cell type. Hybridomas grow well in cell culture and each hybridoma produces a single immunoglobulin. The hybridomas are screened for antigen recognition using immunoassay techniques. Once a hybridoma producing the desired antibody is found, it is cloned for large-scale production. The result is a preparation of a single antibody population. Monoclonal antibodies generally have lower affinities than polyclonal antibodies and are more expensive to produce.

Polyclonal antibodies are obtained from the serum of an immunized animal. In contrast to a monoclonal antibody preparation, polyclonal antiserum contains several different populations of antibodies with varying degrees of selectivity towards the immunogen. The activity of a polyclonal antiserum is a combination of the responses from the different antibodies present. Many environmental immunoassays are based on polyclonal antibodies, because they can have a high affinity for a given analyte. Polyclonal antibodies can be difficult to obtain in large, reproducible quantities; however, large-scale production can be achieved using goats, sheep, or other large animals.

One new theory suggests that antibodies are initially adaptable, and can change shape to accommodate many different targets during their maturation process. However, as antibodies mature, they lose this adaptability and recognize only one antigenic determinant (16). Some researchers are now suggesting that even mature antibodies may perhaps, in some situations, be able to adapt to new targets by changing shape. Whether or not antibodies are capable of adapting to new targets, reaction to nontarget compounds is still possible through cross-reactivity.

**Antibody Mimics and Recombinant Antibodies**

Molecular imprinting enables the production of receptor molecules for select small target analytes. Polymer imprinting is based on the self-assembly of monomers in solution with a template. A cross-linking polymerization process yields a highly durable solid material that selectively binds the template configuration based on shape-selective cavities. Although the concept of molecular imprinting is not new, there is an emerging commercial interest in these reagents. Imprinted polymeric materials are being evaluated as reagents for selective binding applications. Binding affinities for these synthetic receptors have been shown to be similar to specific antibodies.
used in immunoassays. Cross-reactivity profiles have also been similar when antibodies and imprinted polymers are compared in immunoassay formats. Batch-to-batch reproducibility is reportedly high, however, imprinting has yet to be applied to a wide variety of compound classes. Although the existing technology can supply a large amount of an invariant reagent, problems exist in applying the reagents to chromatography, such as slow mass transfer and recognition problems in aqueous media (17). Another application of these reagents could be the study of binding mechanisms of toxic compounds in pharmacokinetic studies of human exposure.

Data Analysis and Quality Assurance Issues

Many classes of environmental contaminants are structurally similar and are likely to appear as co-contaminants in a sample. It is extremely difficult to develop a completely monospecific antibody that recognizes compound A, for example, but not its methyl, ethyl, or dithio analogs. Epitopes, or antibody recognition sites, can also be held in common by 2 or more different compound types. Immunoassays that are developed for specific compounds often recognize structurally similar compounds as well, sometimes making exact quantitation a mathematical and analytical challenge. To estimate this cross-reactivity, it is necessary to determine the levels at which similar compounds affect assay results. Generally, concentrations cannot be determined from linear approximations from an immunoassay standard curve. Nor can assay response be directly related to the sum of the concentrations of individual analytes. One method of addressing this data analysis problem is to use a 4-parameter model to identify the response to cross-reactive compounds and to account for nonspecific binding that might otherwise be attributed to cross reactivity (18). Mixture analysis and pattern recognition may use cross-reactivity as an advantage. Mixture analysis can be performed on assays employing more than one antibody. For these systems, the number of antibodies must be equal to the number of target components in the sample (19). This approach has been used for mixtures containing 4 analytes. Neural networks have also been applied to deconvolute cross-reactivity data.

An evaluation of 5 commercial software systems to determine the correctness and reproducibility of widely-used programs (11) elucidated small variability in results. This evaluation showed there are considerable differences in the number of data analysis options available to users. However, users with only one software package do not have the luxury of performing comparison studies with every analysis. Therefore, it is necessary to include appropriate controls and performance checks with each immunoassay performed.

As with all analytical chemistry projects, a QA program should be implemented. In part, a QA program should include quality control protocols for the immunoassay method, confirmatory method (when one is used), extraction and cleanup procedures, performance checks, instrument calibration and maintenance, appropriate curve fitting models, data handling and analysis, troubleshooting guidelines, and documentation of assay conditions, techniques, and sampling procedures (13).

General guidance concerning sampling and sample preparation for immunoassay analysis, preparation of standards and samples, optimization of reagent concentrations, data analysis, interpretation and interpolation, and the use of curve fitting models is available (13). Table 2 (from *A User’s Guide to Environmental Immunochemical Analysis*, EPA/540/R-94/509) provides troubleshooting guidance for optimizing immunoassay method performance.

Pesticide Applications

Foliage dislodgable residues (FDRs) are of interest to environmental regulators, registered pesticide applicators, farmers, and consumers. Pesticide residues from previously applied treatments can become airborne during the actions of agricultural workers, posing both inhalation and dermal exposure hazards. For these reasons, regulatory agencies have established worker reentry times based on tolerance levels set forth in the Code of Federal Regulations (20).

An indirect inhibition ELISA was developed and applied to the analysis of the organophosphate chlorpyrifos (O,O-di-ethyl-O-[3,5,6-trichloro-2-pyridyl] phosphorothioate) for dislodgable residues from various garden vegetables (21). The ELISA was applied to crude leaf wash samples contained in a dilute surfactant solution (0.0026% sodium dioctylsulfosuccinate). Sample extracts were split for confirmatory analysis by HPLC. Although the ELISA could be performed on the aqueous extracts, the HPLC method required an extraction with ethyl acetate. The ELISA and HPLC were in close agreement within the analysis range of 22–125 ng/mL for apple, tomato, and nectarine leaf washes. The ELISA was easier to perform, required fewer steps, and had a higher throughput. An important attribute of the method is the ability to perform the test in the field for worker protection.

Nonoccupational exposures to pesticides can also pose human health concerns. Track-in dirt and dust-borne pesticides may constitute a significant exposure for crawling infants and toddlers. Older children may inadvertently consume significant quantities of dirt and dust while playing on carpets. Given an identical exposure, children may be at a higher risk than adults because of their developmental state. An immunoassay was developed for chlorpyrifos from track-in dirt (22). Track-in dirt samples were collected from various outside doormats. A sonic extraction with methanol provided a fast and efficient extraction of chlorpyrifos from the dirt samples. The dirt samples (200 mg) were spiked with unlabeled and labeled C14-chlorpyrifos and allowed to stand overnight. Samples were then extracted with 5% methanol (10 mL) and sonicated for 30 min (this step was repeated once). After centrifugation at 10 000 rpm for 30 s, a 1 mL aliquot of each sample was removed and placed in a scintillation counter. Results showed that a 5 min sonication was efficient in dislodging chlorpyrifos from the dirt sample. When PBST (phosphate-buffered saline containing 0.005% Tween 20) was substituted for methanol, chlorpyrifos was only minimally extracted from the dirt samples. Methanol extracts were analyzed by an indirect ELISA. The ELISA format was modified slightly by optimizing the antibody–antigen incubation step.
The standards and primary antibody were mixed together and subjected to sonication. Standard curve IC₅₀ values for 2, 3, 4, 5, and 8 min were very similar. A 5 min sonication time was found to be comparable to an overnight incubation period. A commercial formulation of 6.5% chlorpyrifos (Dursban®) was assayed by the abbreviated method versus an overnight incubation. The two procedures were in close agreement, yielding 7.5 and 7.6% chlorpyrifos, respectively.

ELISA techniques for chlorpyrifos determination are being applied to the analysis of various food commodities for future monitoring studies (23). Chlorpyrifos exposures may also be determined by measuring its urinary metabolite 3,5,6-trichloro-2-pyridinol (TCP). A paramagnetic immunoassay was adapted to determine TCP in urine at low ppb levels (24). The method was used to analyze samples from various exposure surveys. Another TCP immunoassay is being adapted by the NERL-LV for implementation into a multi-year exposure study.

**Other Human Exposure Applications**

Human exposure to potentially harmful substances is not limited to pesticides. The EPA is interested in monitoring exposure to PCBs, benzene, toluene, xylene (BTX), and PCP, among other contaminants. A quantitative ELISA for PCBs in environmental soil and sediment samples was developed and its performance compared with that of gas chromatography (GC) using an electron capture detector (ECD). Very limited cross-reactivity was seen with 37 structurally related co-contaminants. Three extraction procedures were evaluated: an abbreviated methanol shake procedure, supercritical fluid extraction (SFE) with carbon dioxide, and Soxhlet extraction with methanol (25). The samples extracted by SFE and Soxhlet were in close agreement with GC–ECD results. The simple methanol shake method yielded lower ELISA results, supporting the importance of extraction procedures in the overall analysis scheme. The tandem SFE–ELISA technique was also applied to oily soil samples. In a study of 13 soil samples, the ELISA results were essentially the same.

### Table 2. General troubleshooting guidelines for optimizing enzyme immunoassay method performance (13)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor well-to-well replication</td>
<td>Poor pipetting technique</td>
<td>Check instrument, see tutorial on pipetting, practice, calibrate pipettor</td>
</tr>
<tr>
<td></td>
<td>Poor binding plates</td>
<td>Check new lot, change manufacturers</td>
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<tr>
<td></td>
<td>Coating antigen or antibody is degrading</td>
<td>Use new lot of coating reagent</td>
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<td></td>
<td>Coated plates stored too long</td>
<td>Discard plates, coat a new set, decrease storage time</td>
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<tr>
<td></td>
<td>Poor washing</td>
<td>Wash plates more carefully, remake buffer</td>
</tr>
<tr>
<td></td>
<td>Uneven temperature in the wells</td>
<td>Deliver reagents at room temperature, avoid large temperature fluctuations in the room</td>
</tr>
<tr>
<td></td>
<td>Sample carryover</td>
<td>Watch for potential carryover in pipetting and washing steps</td>
</tr>
<tr>
<td>Low or no color development</td>
<td>Loss of reagent integrity</td>
<td>Systematically replace or check reagents, including buffers, beginning with the enzyme label</td>
</tr>
<tr>
<td></td>
<td>Incubation temperature too cold</td>
<td>Lengthen incubation time or increase temperature by using a circulating air-temperature controlled incubator (particularly a problem if working in the field).</td>
</tr>
<tr>
<td></td>
<td>Sample matrix effect</td>
<td>Dilute matrix if possible, check pH of matrix, increase the ionic strength of the buffer</td>
</tr>
<tr>
<td>Color development too high</td>
<td>Incubation too long or temperature too high</td>
<td>Decrease incubation time or temperature</td>
</tr>
<tr>
<td></td>
<td>Matrix effect</td>
<td>Dilute matrix or re-evaluate matrix effects</td>
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<tr>
<td>Change in calibration curve parameters</td>
<td>Degradation of reagents</td>
<td>Systematically check or replace reagents, including buffers</td>
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<td></td>
<td>For short assay times, incubation too long</td>
<td>Monitor incubation times carefully</td>
</tr>
<tr>
<td>High background</td>
<td>Incubation too long, favored nonspecific binding</td>
<td>Monitor incubation times carefully</td>
</tr>
<tr>
<td></td>
<td>Used too high reagent concentrations, favored nonspecific binding</td>
<td>Make sure the correct reagent concentrations are used</td>
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<tr>
<td>High plate-to-plate variation</td>
<td>Poor uniformity of coating</td>
<td>Use new aliquot of coating antigen or antibody</td>
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<tr>
<td></td>
<td>Non-uniform binding plates</td>
<td>Choose new lot of plates</td>
</tr>
<tr>
<td></td>
<td>Poor pipetting technique</td>
<td>Check instrument, see tutorial on pipetting, practice</td>
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as those from GC–ECD analysis on sample splits. These results indicate that this ELISA is an effective quantitative method for PCB determination when used with a rigorous extraction technique.

The EPA’s Office of Underground Storage Tanks has identified more than 2 million active or closed regulated storage tanks in the United States. The most frequently found regulated contaminants from leaks in these tanks are gasoline and petroleum products which frequently contain BTX compounds. A field evaluation of an ELISA for BTX compounds was performed and results were evaluated against GC results (26). The immunoassay was not as sensitive as the manufacturer claimed but it performed well on samples containing more than 200 ng/g BTX. By slightly adjusting the response level, the ELISA could be used to classify samples between 25 ng/g BTX with few false-positive results.

Numerous hazardous waste sites regulated under Superfund contain PCP, a chemical used in agriculture and as a wood preservative. An immunoassay developed to detect trace levels of PCP in water was evaluated on drinking water, ground water, and surface water samples using 3 different methods: solid-phase extraction (SPE) cartridges with ELISA, EPA Method 604, or direct ELISA without any extraction step (27). The immunoassay was also evaluated for cross-reactivity. The immunoassay accuracy was best for drinking water samples, especially when the assay was preceded by either of the extraction steps. The major cross-reactants were 2,3,5,6-tetrachlorophenol and 2,4,6-trichlorophenol.

Cleanup Technologies

Immunospecificity chromatography (IAC) exploits the ability of antibodies to separate analytes that are present in complex matrices. In high performance immunospecificity chromatography (HPIAC), a specific antibody is immobilized onto an LC support and used as an affinity ligand. The antibody selectively extracts the analyte from an aqueous sample extract injected onto the HPIAC column. Immunospecificity chromatography minimizes reliance on organic solvents to achieve efficient separations prior to ELISA or instrumental analysis.

A selective SPE by immunoaffinity capillary electrophoresis (IACEC) was used in a model system prior to analysis by capillary zone electrophoresis (CZE) with laser induced fluorescence (LIF) detection (28). The analyte, FITC-biotin, was electrokinetically applied to a capillary column packed with an immobilized anti-biotin-IgG support. After selective extraction by the immunospecificity capillary, the bound analyte was eluted, migrated by CZE, and detected by LIF. The column was regenerated several times for reuse.

The calibration curve for bound FITC-biotin versus application time was linear from 10 to 300 s. Recovery of FITC-biotin spiked into a dilute urinary metabolite standard solution was 89.4% relative to spiked buffer, with a precision of 1.8% relative standard deviation (RSD). This system may have application for selective trace enrichment of low level analytes of toxicological significance prior to detection by CZE with LIF detection.

Hyphenated Techniques

Hyphenated techniques, such as SFE–ELISA and HPIAC–LC with mass spectrometry (MS), build on the advantages of 2 or more methods to provide a modular approach to difficult analyses. By coupling IAC to a powerful detection system, such as LC/MS or capillary electrophorography–LIF, it is possible to optimize an analytical procedure using both traditional and antibody-based methods (29). By coupling HPIAC with LC, it is possible to achieve a 2-dimensional separation, combining the selectivity of immunospecificity with the resolution power capable of separating structurally similar compounds (30).

An application of the tandem HPIAC–LC approach is the analysis of carbendazim (30). The method allowed the on-line extraction, preconcentration, and positive confirmation of carbendazim with a throughput of one sample every 10 min. The method had a linear range of 0.025–100 μg/L for UV-Vis diode array detection. This tandem system can be adapted for other classes of pollutants when used with other antibodies and columns.

Sensor Technologies

Immunospecificity methods are slowly making inroads into the field of environmental sensors. Biosensors are analytical tools consisting of a biological recognition element and a signal transducer that translates the biological event into a quantifiable signal. The ideal sensor provides continuous and selective monitoring data and is easily regenerated. The majority of bio sensor research is currently directed towards clinical applications. However, interest and research into environmental biosensors are increasing. When the recognition element of a sensor is an antibody, the sensor can be called an immunosensor. The following examples illustrate some of the immunosensors currently being developed for environmental applications.

Immunosensors are typically based on either optical or electrochemical detection. A fiber-optic immunosensor (31) and a continuous flow immunosensor (32) to detect explosives have been developed by the Naval Research Lab. The fiber-optic sensor has a minimum detection limit of 8 ppb for TNT in a variety of water media. The continuous flow immunosensor was field tested at an EPA Superfund site where it was shown to be an attractive alternative to HPLC. Both of these sensors use fluorescent analogs of the analytes of interest for detection. Surface plasmon resonance (SPR) sensor technology is used to characterize and quantitate biomolecular interactions and can also be used for real-time monitoring and remote sensing devices (33). An inhibition immunoassay was coupled to a commercially available SPR sensor chip technology for determining atrazine (34). The SPR response changes, due to the competition of antibody for atrazine in the sample and for the atrazine derivative covalently bound to the sensor chip, enabling quantitation. A detection limit of 0.05 ppb in water can be achieved with an analysis time of 15 min (34). Sol-gel-derived thick-film amperometric immunosensors have been developed for im-
mumoglobin detection (35). The technique may eventually be applied to compounds of environmental significance.

An electrochemical immunosensor based on a conducting polymer-modified electrode has been developed for PCB monitoring (36). An anti-PCB antibody is immobilized within a polypyrrole matrix on the surface of an electrode. Using a pulsed amperometric waveform, the antibody–analyte interaction is either encouraged (generating a signal) or discouraged (regenerating the sensing surface). A “disposable” electrochemical immunosensor format for field monitoring is being developed based on screen-printed electrodes. A competitive immunoassay for 2,4-dichlorophenoxyacetic acid (2,4-D) incorporates an immobilized antigen complex at the surface of a screen-printed working electrode for the quantitation of the herbicide in methanolic soil extracts (37). The single-use sensor compared favorably with a commercial 2,4-D immunoassay test method and required fewer manipulations. Atrazine has also been detected using disposable screen printed electrodes. Electrodes modified with horseradish peroxidase were used as the detector element in a competitive immunoassay (38). This electrochemical immunosensor also compared well with a commercial immunoassay test method. An amperometric immunosensor based on sequential injection analysis was developed for 2,4-D (39). The system achieved a sensitivity in the range required for the European Drinking Water Regulation. The above sensor technologies may provide automated systems to support regulatory compliance, pollution prevention measures, and other monitoring requirements.

Regulatory and User Acceptance Issues

Several facets of immunoassays make them particularly suitable for screening large numbers of samples in a field setting, be it a farm, factory, or Superfund site. Many immunoassays are easily portable to the site, easily learned by nonscientist users, less expensive than traditional methods, provide results in minutes, and tend to have a positive bias due in part to cross reactivity. In environmental practice, false-negative results are not acceptable but false-positive results, while not ideal, may provide an extra measure of protection. As analytical chemistry moves from traditional laboratory settings to mobile labs, and ultimately to field use, there will be increased demand for recognition of screening methods. In 1996, immunoassay methods were included in the EPA’s Office of Solid Waste methods compendium, SW-846 (40).

Regulatory acceptance is critical to the successful incorporation of new methods in all agencies. In the past few years, there has been growing acceptance by the EPA of methods that are based on reasonable results. These performance-based methods can replace detailed prescriptive methods when sufficient data substantiate their comparability with established methods, allowing for flexibility. Performance-based protocols may be instrumental in recognizing alternative methods such as immunoassays.

Future Applications

The versatility of immunochemical techniques makes them suitable to applications like personal exposure monitors, where exposure to airborne chemicals would result in a colorimetric change. These devices would alert the wearer to potential risk rather than record that an exposure has occurred. Other immunochemical methods have application for the detection of airborne bacterial spores and toxic molds. Methods that can be easily performed in home environments will help to identify individuals who are at risk of exposure.

New analytical methods and instrumentation are bridging the gulf between immunochemical and instrumental methods (Table 3). A shortcoming of immunoassay may be the inability to distinguish between structurally related compounds or cross-reactive co-contaminants. The on-line coupling of continuous flow immunochemical detection methods with HPLC combines the resolving power of HPLC with the sensitivity of antibodies. Perhaps successful analysis of cross-reactive compounds in complex matrices may eventually be accomplished using this approach.

Immunochemical methods for metals have been slower in development than those for organic compounds. There are at least 20 toxic metals and several of these, including arsenic, cadmium, chromium, copper, lead, nickel, silver, selenium, and zinc, are present in the environment at levels high enough to present human exposure problems (46). The use of metal-chelates, typically metal ion–EDTA chelates, makes immunochemical analysis of metals feasible (47). However,
these methods need to compete effectively with techniques that can speciate.

Conclusions

The use of immunochemical technologies in environmental monitoring and human exposure assessment studies continues to expand on 3 levels. First, the regulatory communities have continued to accept innovative methods that have established their reliability in real-world applications. Second, researchers are constantly developing more sensitive and more specific methods for immunochemical determination of environmental contaminants. Third, first-generation immunoassay users, satisfied with analytical results, are beginning to rely on these methods which were novel only a decade ago.

As the need for rapid, low-cost screening methods continues in hazardous waste site remediation, food safety inspections, and large-scale ecological and human exposure monitoring, the use of immunochemical technologies will expand. These new applications will be followed by increased recognition and acceptance.

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References


