Environmental Immunoassays and other Bioanalytical Methods: Progress and Prospects

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1. Introduction

Current interest in novel, cost effective analytical methods for environmental contaminants has two main motivations. First is the need to improve efficiency because of rising costs and, not uncommonly, cuts to program funds. Second is the need to better understand the sources, distribution, fate, and effects of a host of agrochemicals and industrial pollutants many of which are ecologically harmful. The challenge is formidable since the trace level analysis of many contaminants of environmental matrices is costly and time consuming.

It is not uncommon for a large proportion of samples from monitoring studies to be analyte free or to contain undetectable or meaningless levels of analyte. A screening strategy that could identify those negative samples for elimination from the sample set, and help to prioritize the positive samples, would help to reduce costs and improve analytical services. Bioanalytical methods can be used either as part of a screening strategy, or as analytical tools in their own right, for the cost effective detection of important analytes. Most bioanalytical methods are based on the ability of key biological molecules, such as antibodies or receptors, to recognize a unique structural property of the analyte, or on the ability of cells or organisms to respond diagnostically to some contaminants. The two groups of bioanalytical methods can for convenience be termed "ligand binding assays" and "bioassays". The ligand binding assays include immunoassays (IAs) and receptor binding assays (RBAs). The bioassays include the various multi function oxygenase (MFO) induction assays.

2. Immunoassays

IAs have proven their worth in the clinical laboratory where they helped to transform analytical strategies. IAs exploit the ability of specialized biological molecules, called *antibodies* (ABs), to selectively and reversibly bind organic molecules. The other key reagent in most environmental IAs is the labelled ligand, or depending on the assay format, the coating antigen. IAs owe their versatility to the immune system's ability to produce ABs in response to virtually any foreign organic molecule. For small analytes, the compound of interest must be conjugated to a large carrier molecule, such as BSA, to render it immunogenic. The site of conjugation and the linkage can influence the specificity and sensitivity of the final assay.

The ligand molecules can be labelled with a radioactive tracer, an enzyme, or a fluorescent molecule, so that the fraction of ligand molecules that has been bound can be estimated. Again, the linkage design can profoundly influence the resulting assay. Most environmental IAs are competitive binding assays in which the binder molecule, an excess amount of labelled

analyte, or coating antigen, and the target analyte are allowed to approach equilibrium. The free analyte is then separated from the bound phase and the amount of labelled analyte, or binder molecule, that has been bound is quantified. The amount of analyte in the unknown samples is interpolated from a calibration curve. The details of the various assay formats can vary but the competitive binding concept remains the same.

Most modern IAs for ecosystem contaminants use enzyme tracers to provide the quantification signal. The long shelf lives, ease of distribution, and suitability for field use of enzyme based IAs (EIAs) make them attractive to environmental analysts. Enzyme based assays have adequate sensitivity for most purposes, and have been developed in a variety of configurations.

3. Advantages and disadvantages

Some advantages and disadvantages of environmental IAs are summarized in Table (1)[1,2]. A growing consensus favours a role for IAs in screening strategies and in niche applications, such as the measurement of biomarkers. Field analysts have long wished for portable methods that are both simple and reliable. AB based techniques can fill that role. IAs can be used to select sites that warrant more intensive study, to alleviate the problem of sample stability, and to survey or monitor effluents or contaminated waters. IAs can help provide the timely data that are often needed when dealing with industrial spills and environmental hot-spots.

Table 1. Some advantages and disadvantages of IA screening techniques for environmental analytes.

Advantages	*Disadvantages*
Sensitive and Specific	Development costly
Rapid and Easy to use	Hapten synthesis can be difficult
Cost-effective	Can be vulnerable to cross reacting compounds
Small sample size	and non-specific interferences
Ease of use	Requires independent confirmation
Wide applicability	Not suited to small sample loads or multi-residue
Reduced preparation	determinations
Simultaneous analysis of multiple samples	Lack of familiarity, conservative attitudes
Ideal for large loads and easily automated	
Suited to field use	

4. Environmental IAs

Ercegovich introduced IA techniques for ecosystem contaminants in the early 70's[3]. Numerous IAs and related techniques have since been developed for a broad range of pesticides and contaminants of industrial origin[1,2,4]. Among the early IAs for environmental analytes that influenced and inspired much subsequent research is a set of assays for chlorinated planar organic molecules including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) (Table 3) that was developed by Albro and co-workers at the National Institute for Environmental Health Sciences.

The ability of many IAs to reliably detect their targets in environmental matrices has been validated, and several of the assays have been used in environmental studies[1,5,6]. When used as part of a screening strategy, IAs are intended to complement, not replace, conventional

techniques. After the samples have been screened, the positive samples and a random selection of negative samples should be confirmed by an independent technique, such as gas chromatography (GC) or high performance liquid chromatography (HPLC).

5. IAs for industrial chemicals

Table 2 describes some IAs for industrial analytes. Interest in a screening strategy for halogenated aromatic hydrocarbons (HAHs) was, and still is, stimulated by the high cost of determining those analytes. Dioxins are particularly expensive to determine: ranging from CAN$1000 - CAN$1500 per sample. Both RIAs and EIAs have been developed for the detection of PCDDs and PCBs. Stanker et al.'s development of a suite of MABs for PCDDs provided a source of well defined ABs upon which future assays, probes, or techniques can be based[7]. Most interest in IAs for PCBs has focused on the various Aroclors as target analytes. Commercial EIAs for the detection of PCBs are available from the following companies: ImmunoSystems (Scarborough, ME), EnSys, Inc. (Research Triangle Park, N.C.), and Ohmicron (Newborn, P.A.). IAs have also been used to detect PAHs such as benzo[a]-pyrene[8] and their DNA[9] or protein[10] adducts.

6. Acceptance and implementation of IAs

Although IAs are being increasingly used to detect water soluble contaminants of water, soil and food, acceptance of their use for the detection of hydrophobic analytes, such as the HAHs, has lagged. There are two reasons for this. In the absence of a threshold level below which samples can be considered negative, the desired detection limit for HAH analytes has been driven ever lower by each generation of mass spectrometers. Because those DLs are far less than current IAs can achieve, the IA screening option cannot yet reliably identify analyte free samples in many situations. An improvement in assay sensitivity, or the acceptance of threshold levels within the working range of the IA would solve that problem. IAs can, of course, be used to identify highly contaminated samples such as may be found at industrial sites.

Sample preparation costs are an issue when IAs or RBAs are used to detect hydrophobic compounds, such as the HAHs, in oily matrices. Because the binder-ligand reaction occurs in the liquid phase, the analyte molecules must first be extracted from the matrix, separated from the lipid phase, and then solubilized in aqueous buffer. The extra steps can diminish the practical advantages of a screening test. Efficient extraction techniques such as super critical fluid extraction (SFE) and solid phase extraction (SPE) can simplify the preparation of samples for immuno-analysis[12,13]. Water samples would probably need less cleanup.

Several challenges must be met if IAs are to make the transition from research tools to widely used analytical methods. Individual IAs must meet clearly defined, and widely accepted performance criteria. The approval of IA screening tests by major agencies, or the implementation of "Flagship Assays" in some high profile programs would enhance the credibility of IA screening tests. Continued improvements in the distribution and accessibility of IA reagents will encourage new users to become involved. There is also a need to promote an understanding of IA screening strategies within the wider analytical community including an awareness of the benefits and shortcomings of IA screening techniques.

Table 2. Some IAs for industrial chemicals.

Analyte	Format	Range	DL	I_{50}	Reference
PCDDs 2,3,7,8-T_4CDD	RIA[^{125}I]	F_{12}Cutscum: 50 pg - 75 ng GC_5 Cutscum: 200 pg - 20 ng	25 pg	20 ng 4 ng	1)
PCDDs 2,3,7,8-T_4CDD	RIA[^{3}H]	GC_5 DMSO: a: 20 pg - 2 ng b: 2.5 pg - 200 pg	21 pg 3.9 pg	350 pg 42 pg	1)
PCDDs 2,3,7,8-T_4CDD	EIA	40 pg - 10 ng	100 pg	1 ng	1)
PCBs	RIA[^{125}I]	Aroclor 1260: 100 pg - 3 ng	100 pg	400 pg	1)
PCBs	RIA[^{125}I]	Aroclor 1260: 125 pg - 5 ng	100 pg		1)
PCBs	EIA	Aroclor 1254: 0.2 - 10 ppb in water	200 ppt		1)
2,4,6-trinitrotoluene	EIA	1 - 30 μg/L		4.5 μg/L	11)

7. Other bioanalytical techniques for HAHs

The various bioassays for the detection of HAH like compounds are based on the ability of many dioxin-like planar aromatic hydrocarbons to interact with the Ah receptor thus inducing the synthesis of characteristic proteins, such as the cytochrome P450 based MFOs, or the alteration of cellular characteristics. MFOs have been used as the response parameter in *in-vitro* assays, or as bioindicators of *in-vivo* exposure in either wild or test animals. The induced MFOs can be measured enzymatically or by EIA[14].

The bioassays effectively integrate a sample's planar aromatic hydrocarbons in direct proportion to their ability to bind to the Ah receptor. Since the chronic toxicity of dioxin-like compounds is believed to be mediated by the Ah receptor, the bioassays can be used to estimate a sample's potency, which is usually expressed as 2,3,7,8-TCDD equivalents (TEQs). With the possible exception of the flat cell bioassay[15] which is reported to be most sensitive to the more toxic PCDD/PCDF congeners, the various bioassays tend to cross-react with any molecule that can bind to the Ah receptor. Some bioassays for the detection of dioxin like molecules are listed in Table 3. *In-vitro* assays that use either primary liver hepatocyte cells or a cell line that retains the ability to produce MFOs are convenient to use, albeit a little less realistic. The *in-vitro* assays are sensitive, are readily automated and miniaturized[16], and could probably be commercialized.

Receptor binding assays (RBAs), which are similar in principle to the competitive binding IAs have been developed for the detection of Ah active compounds[17] and have potential use in the detection of compounds that can bind to the estrogen receptor.

8. Prospects

Among the emerging immuno-techniques that are likely to be useful to analysts are integrated immuno-probes for the detection of analytes[18]; immunoaffinity chromatography techniques for the isolation of targeted analytes from environmental matrices[19]; and flow injection immuno-

analysis (FIIA) techniques for the rapid measurement of analytes in small volume samples[20]. Ekins[21] has outlined a multi-analyte IA system which if brought to fruition could revolutionize IA technology by permitting the simultaneous analysis of multiple analytes at ultra low detection limits.

Bioanalytical techniques are poised to play a high profile role in environmental science by providing biologically relevant estimates of contaminants in environmental samples, and by helping to detect and identify harmful chemicals. The bioanalytical approach helps to link conventional analytical chemistry to complex biological and ecological processes.

Table 3. Some bioassays for dioxin-like compounds.

Organism	Type	Parameter	Linear Range	ED_{50} (TCDD)	Reference
Rainbow trout	*in-vivo*	EROD		700 pg TCDD/g liver (200 g fish (i.p. exposure)	[22]
Chicken embryo hepatocytes	*in-vitro*	EROD	10^{-3} nM - 10^{-1} nM	15 pM	[23]
Rat H-4-11-E	*in-vitro*	EROD	31.6 - 251 pg/plate	0.17 pmol/plate	[24]
Rainbow trout	*in-vitro*	EROD	2.5 - 100 pM		[25]

9. References

1) Sherry J.P. (1992): Environmental chemistry - the immunoassay option. CRC Crit. Rev. Anal. Chem. 23, 217-300
2) Vanderlaan M., B.E. Watkins, and L. Stanker (1988): Environmental monitoring by immunoassays. Environ. Sci. Technol. 22, 247-254
3) Ercegovich C.D. (1971): Analysis of pesticide residues: immunological techniques. In: Pesticide Identification at the Residue Level, Gould R.F., ed., American Chemical Society, Washington, D.C., pp. 162-177
4) Van Emon J., J.N. Seiber, and B.D. Hammock (1989): Immunoassay techniques for pesticide analysis. In: Analytical Methods for Pesticides and Plant Growth Regulators, Sherma J., ed., Academic Press Inc., New York, N.Y., pp. 217-263
5) Sherry J.P. (1995): Immunodetection of ecosystem contaminants: research, application, and acceptance in Canada. In: Immunoanalysis of Agrochemicals: Emerging Technologies, Nelson J., A.E. Karu, and R.B. Wong, eds., American Chemical Society, Washington, pp. 335-353
6) Zajicek J.L., R.O. Harrison, and D.E. Tilitt. Enzyme-linked immunosorbent assay (ELISA) compared to high resolution gas chromatography (HRGC) for measurement of PCBs in selected fish extracts. Presented Eighth IUPAC International Congress of Pesticide Chemistry, July 4-9, Washington, DC, Presentation # 66.
7) Stanker L.H., B. Watkins, N. Rogers, and M. Vanderlaan (1987): Monoclonal antibodies for dioxin: antibody characterization and assay development. Toxicology 45, 229-243
8) Roda A., A. Pistillo, A. Jus, C. Armanino, and M. Baraldini (1994): Analysis of air particulate benzo[alpha]pyrene by a specific enzyme immunoassay: Correlation with chemical and atmospheric parameters. Anal. Chim. Acta. 298, 53-64
9) Vo-Dinh T., J.P. Alarie, R.W. Johnson, M.J. Sepaniak, and R.M. Santella (1991): Evaluation of the fiber-optic antibody-based fluoroimmunosensor for DNA adducts in human placenta samples. Clin. Chem. 37, 532-535

10) Lee B.M., R.M. Santella (1988): Quantitation of protein adducts as a marker of genotoxic exposure: immunologic detection of benzo[a]pyrene-globin adducts in mice. Carcinogen. 9, 1773-1777
11) Tomita M., K. Suzuki, K. Shikmosato, A. Kohama, and I. Ijiri (1988): An enzyme-linked immunosorbent assay for plasma paraquat levels of poisoned patients. Forensic Science International 37, 11-18
12) Lopez-Avila V., C. Charan, and W.F. Beckert (1994): Using supercritical fluid extraction and enzyme immunoassays to determine pesticides in soils. Trends Anal. Chem. 13, 118-126
13) Aga D.S., E.M. Thurman (1993): Coupling solid-phase extraction and enzyme-linked immunosorbent assay for ultratrace determination of herbicides in pristine water. Anal. Chem. 65, 2894-2898
14) Goksoyr A., A.M. Husoy (1992): The cytochrome-P450 1A1 response in fish - application of immunodetection in environmental monitoring and toxicological testing. Marine Environ. Res. 34, 147-150
15) Gierthy J.F., D. Crane (1986): Development and application of an *in vitro* bioassay for dioxin-like compounds. In: Chlorinated Dioxins and Dibenzofurans in Perspective, Rappe C., G. Choudhary, and K. Keith, eds., Lewis Publishers Inc., Michigan, pp. 269-284
16) Kennedy S.W., A. Lorenzen, C.A. James, and B.T. Collins (1993): Ethoxyresorufin-o-deethylase and porphyrin analysis in chicken embryo hepatocyte cultures with a fluorescence multi-well plate reader. Anal. Biochem. 211, 102-112
17) Bunce N.J., R. Logan, and U.A. Schneider (1990): Development of a rapid screening assay for PCDDs and PCDFs. Chemosphere 20, 1417-1422
18) Bier F.F., R. Jockers, and R.D. Schmid (1994): Integrated optical immunosensor for s-triazine determination: regeneration, calibration and limitations. Analyst 119, 437-441
19) Rule G.S., A.V. Mordehai, and J. Henion (1994): Determination of carbofuran by on-line immunoaffinity chromatography with coupled-column liquid chromatography/mass spectrometry. Anal. Chem. 66, 230-235
20) Nilsson M., G. Mattiasson, and B. Mattiasson (1993): Automated immunochemical binding assay (flow-ELISA) based on repeated use of an antibody column placed in a flow-injection system. J. Biotechnol. 31, 381-394
21) Ekins R., F. Chu, and E. Biggart (1989): Development of microspot multi- analyte ratiometric immunoassay using dual fluorescent-labelled antibodies. Anal. Chim. Acta. 227, 73-96
22) Hodson P.V., S. Efler, M.M. Maj, and J.Y. Wilson. A refined protocol for measuring the potency of effluents, extracts or pure compounds for inducing MFO activity of fish. Poster Presented at 2nd International Conference on Fate and Effects of Bleached Kraft Mill Effluents, Vancouver, B.C. Canada.
23) Kennedy S.W., A. Lorenzen, C.A. James, and R.J. Norstrom (1992): Ethoxyresorufin-o-deethylase, (EROD) and porphyria induction in chicken embryo hepatocyte cultures - a new bioassay of PCB, PCDD, and related chemical contamination in wildlife. Chemosphere 25, 193-196
24) Tillitt D.E., J.P. Giesy, and G.T. Ankley (1991): Characterization of the H411E rat hepatoma cell bioassay as a tool for assessing toxic potency of planar halogenated hydrocarbons in environmental samples. Environ. Sci. Technol. 25, 87-92
25) Pesonen M., A. Goksoyr, and T. Andersson (1992): Expression of cytochrome P-450 1A1 in a primary culture of rainbow trout hepatocytes exposed to beta-naphthoflavone or 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Biochem. Biophys. 292, 228-233