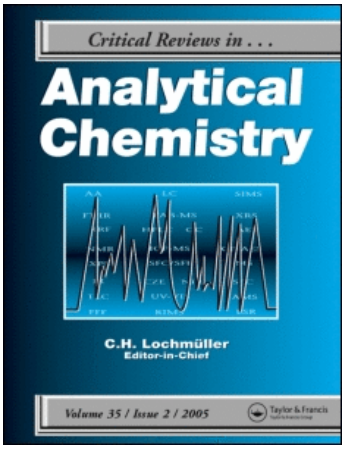

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Environmental Chemistry: The Immunoassay Option

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Environmental Chemistry: The Immunoassay Option

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ABSTRACT: Scientific awareness and public concern about the state of our environment have led to unprecedented demands on analytical laboratories. There is now much interest in strategies that will help to lower costs and improve efficiency. Immunoassay (IA) techniques, which are widely used in clinical chemistry, could play a key role in the laboratory of the future. IA screening techniques for the detection of a broad variety of pollutants, including pesticides and industrial contaminants, are reviewed. IAs are best suited to the analysis of large sets of samples. A majority of the methods are sufficiently sensitive and selective for environmental applications. Some approaches that might help improve the sensitivity of IAs so that ultra-trace analytes can be detected are considered. Analytes can usually be detected in water samples with little or no sample preparation. In many cases, an extraction step, which is sometimes followed by a simple clean-up step, suffices for solid matrices. Hydrophobic analytes, such as the polychlorinated dibenzo-*p*-dioxins, are the exception. Recent advances in the validation of environmental IAs, which should improve the confidence of the unfamiliar analyst, are reviewed. IA kits are available for analysts without the resources to develop their own assays. Some interesting trends that are reviewed include the use of immunosensors for the direct detection of contaminants, the use of immunoaffinity chromatography for the one-step purification of analytes, and the environmental application of electrochemical and flow injection immunoassays. IAs have the potential to boost productivity and release costly instruments for use in the quantification of positive samples.

KEY WORDS: immunoassay; radioimmunoassay (RIA), enzyme immunoassay (EIA), ELISA, residue, contaminant, pesticide, herbicide, insecticide, fungicide, halogenated aromatic hydrocarbons, screen, environment, aquatic environment, immunosensors.

I. INTRODUCTION

Interest in immunoassay (IA) techniques for the detection of environmental contaminants has grown steadily over recent years. IAs are selective and sensitive tests that exploit the immune system's ability to produce antibodies (ABs) in response to virtually any organic molecule. IA technology originated in the late 1950s and is a blend of techniques from analytical and clinical chemistry.[1,2] All IAs are based on the selective and sensitive antibody (AB)–antigen (AG) reaction.

In a typical competitive binding IA (Figure 1), a labeled AG is incubated together with ABs to the target analyte. The label can be a radioisotope, an enzyme, a fluorescent chemical, or any convenient and measurable marker. In some IA formats, the AB rather than the AG is labeled. A portion of sample, or buffer that contains a known amount of analyte, is then added to the assay tube. The labeled and unlabeled analytes compete for highly specific binding sites on the ABs. After an adequate incubation period, the bound and free phases are separated, and the amount of analyte that has been bound by the ABs is quantified. The amount of bound label in each analytical tube is compared with that of a set of analyte-free reference tubes. A reduction in the amount of bound label is inversely proportional to the amount of analyte in the assay tube. The quantity of analyte in the sample can be interpolated from a calibration curve (Figure 1).

Rapidity, ease of use, low cost, and the ability to process large numbers of samples at the same time ensured the widespread use and appreciation

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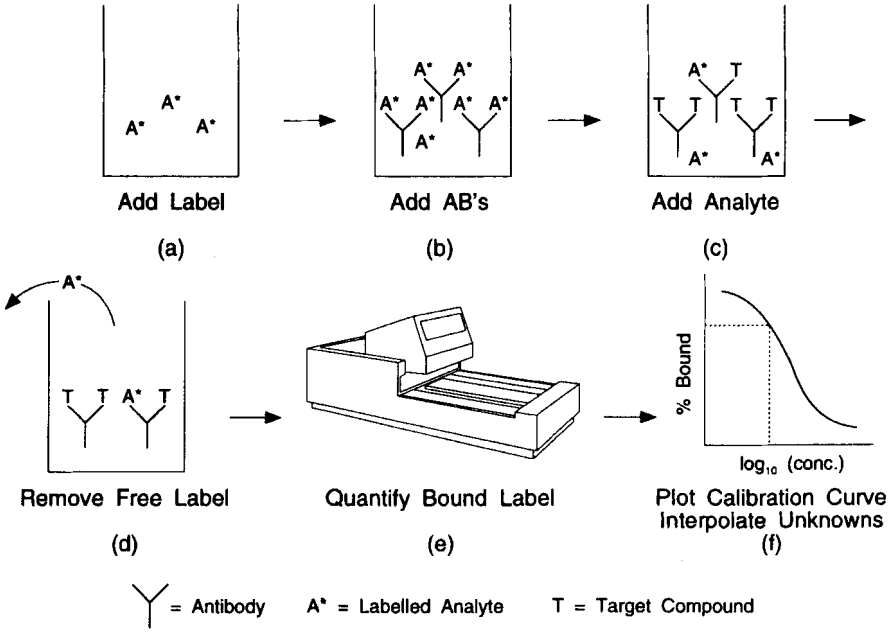


FIGURE 1. Steps in a typical competitive binding immunoassay. Y = antibody; A* = labeled analyte; T = target compound.

of IAs in clinical laboratories. This ready acceptance was driven by the need to determine a wide range of analytes in huge numbers of tissue and fluid samples. For the same reasons, the automation of analytical systems also was pioneered in the clinical laboratory. Environmental laboratories are now faced with similar challenges to those of their clinical counterparts. The present review suggests that IA techniques can transform many difficult and costly routine environmental analyses, thus reducing sample overloads and lowering overall analytical costs. After a slow start, we are witnessing the emergence of a new generation of exciting assays and support techniques that bode well for IA screening tests as a viable option in environmental chemistry. The present report describes the more popular IA techniques and then reviews IA methods for the detection of a variety of environmental contaminants and related compounds. Close attention is paid to IAs that can detect contaminants of the aquatic environment.

A. Background

Yalow and Berson were awarded the Nobel Prize for their development of a quantitative immunological assay of human insulin that made possible the accurate measurement of picogram levels of the hormone in small samples of body fluid.[3] The technique was based on the ability of insulin to displace [^{131}I]-labeled beef insulin from AB-insulin complexes. The displacement of tracer was proportional to the amount of unlabeled insulin. The evolution of IAs as routine analytical tools ensued. The main advantage of the IA over other binding assays follows from the ability of the immune system to produce, on demand, antibodies to virtually any organic molecule. Some 3 decades after their invention, IAs are widely used in clinical laboratories because they are sensitive, specific, and cost effective.[4-6] IAs have been developed for many analytes, including pharmaceuticals, parasites, and bacteria.[6-10]

Antibodies are the key IA reagent, and they, more than the other reagents, determine an assay's characteristics. The antibodies used in IAs (Figure 2) belong to the immunoglobulin's (IgG) gamma fraction and are produced by mammalian lymphocyte B cells, usually in conjunction with T-helper cells, as part of the immune system's response to foreign substances.[11-17] Each differentiated B-cell clone (plasma cells) secretes a single antibody type. Inasmuch as the divalent ABs can form in response to each of the immunogen's antigenic sites, the resultant serum is a mixture (poly-) of clonal antibodies. The AB-AG interaction is weak and involves only noncovalent bonds: Van der Waals interactions, electrostatic bonds, hydrogen bonds, and hydrophobic bonds. The first two bond types

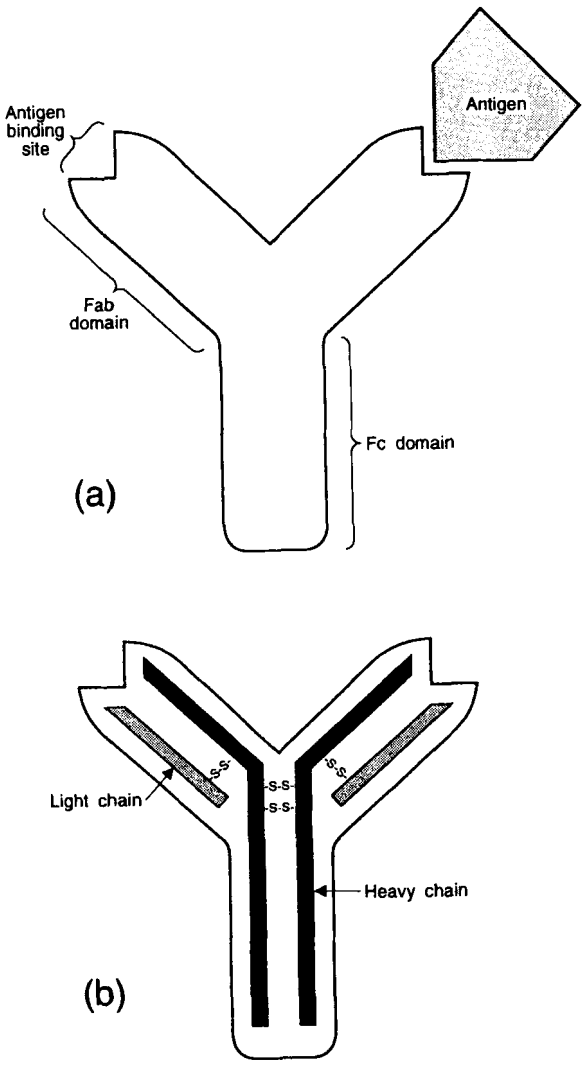


FIGURE 2. Regions (a) and structure (b) of IgG molecules. (Reprinted with permission from Harlow, E. and Lane, D. *Antibodies — A Laboratory Manual.* Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1988.)

usually predominate. Since AB-AG interactions occur over short distances, a close steric fit combined with an accurate match of oppositely charged ions will promote binding.[18] It is these stringent binding requirements that make ABs and IAs so selective. In general, only molecules that contain the matching antigenic determinant, or a very close relative, will bind to an AG-binding site.

AB-AG binding depends on the physical and chemical properties of the reactants, and obeys the law of mass action:[4,19,20]

$$AB + AG \gtrless ABAG$$
$$K = [ABAG]/[AB] + [AG]^{4,19,20}$$

K is a measure of a serum's avidity for AG, or in the case of monovalent MABs, of an ABs affinity for its AG. For some serum-AG combinations, the mass action equation may oversimplify matters since it averages many heterogeneous interactions between the various PABs (polyclonal antibodies) and their antigenic determinants.[18]

1. Immunogens

Most organic pollutants of current interest are small molecules [molecular weight (MW) < 1000] that must be conjugated to a larger carrier molecule if they are to elicit an immune response. The term "hapten" is used to describe such compounds. Bovine serum albumin (BSA; MW 70,000) is a popular carrier;[21] it has plenty of free NH_2 groups and is easily solubilized. Other proteins also can be effective (Table 1); however, the carrier protein must be from a different species than the host animal if a strong immune response is to be induced. ABs can be generated to haptens that are as small as 150 MW .[22]

Often, the target compound contains a functional group that can be covalently coupled to the carrier molecule (Table 1). If not, a suitable group (NH_2, COOH, OH, SH, CO, or HCO) can be introduced into the hapten. In either case, care should be taken to avoid the masking of characteristic features,[16] which could adversely affect antibody specificity. ABs are usually most specific for parts of the molecule furthest from the site of conjugation,[23,24] and lowest for adjoining sites.[10,25] Consequently, the choice of target sector can determine whether the assay will be selective for a class of compounds, a particular contaminant, or a structural subunit. Thus, the design and preparation of the immunogen can have a major influence on an assay's characteristics. Separation of the hapten from the carrier protein by a linkage arm can help improve recognition of the hapten by the host's immune system.[4,16,26] Good antibody titers have been obtained using hapten conjugation ratios of 8 to 25,[26] although there is no consensus as to which ratio is best.[4,24,27] Satisfactory immune responses have been reported for conjugation ratios as low as 2 to 6 haptens per protein molecule[24] and as high as 740.[28] It has been suggested that low substitution ratios are less likely to induce the production of low-affinity ABs.[24]

TABLE 1
Immunogen Preparation

Analyte	Reactive group	Coupling pathway	Ref.
PCBs	4-NH_2-4-MCBP 2-NH_2-4,5,3′,4′-TCBP 3-NH_2-2,6,2′,6′-TCBP	Convert to adipamide using monomethyl adipoyl chloride; hydrolyze to acid; form mixed anhydride with isobutyl chloroformate and couple to protein (Mixed Anhydride Path); BSA/TYG	125
PCBs	2-NH_2-2′,4,4′,5,5′-PCBP	Convert to amide using succinic anhydride; couple to HSA using EDC[a]	160
4-Acetamidobiphenyl N,N′-diacetylbenzidine	4-NH_2-BP 4′-acetamido-4-NH_2-BP	Convert to 4-hemisuccinamido-BP using succinic anhydride; couple using carbodiimide (CDI); BTYG	162, 163
PCDDs	1-NH_2-3,7,8-TCDD	Convert to adipamide; couple using Mixed Anhydride Path; BSA/RSA/TYG	29, 152, 153
PCDDs	2-NH_2-3,7,8-TCDD	Convert to adipamide; couple using Mixed Anhydride Path; BSA; TYG	151
2,3,7,8-TCDF	4-NH_2-2,7,8-TCDF	Convert to adipamide; couple using Mixed Anhydride Path; BSA/TYG	124
4-Nitrophenol	2-hydroxy-5-nitrobenzyl bromide	Nucleophilic substitution; BSA	169
Benzo-a-pyrene	6-NH_2benzo-a-pyrene	BSA	164
2,4-D	2,4,-Cl_2-5-NH_2-D	Convert to R-N_2^+ and couple directly to BSA (Diazonium Path)	170
2,4-D	-COOH	Mixed anhydride method; HSA, BSA, HGG, BGG	172
2,4-D	-COOH	Convert to active ester using NHS[b] and DiCC[c] and couple to protein (Active Ester Path); BSA	174, 230
Picloram	-COOH		
2,4-D	(a) -COOH (b) 2,4,-Cl_2-5-NH_2-D	(a) Active Ester Path (b) Reduce to form amino derivative; Diazonium Path; BSA, TYG	171
Atrazine	R-NH$(CH_2)_5$COOH	Prepare amino caproic acid derivative from cyanuric acid; conjugate via Mixed Anhydride Path; BSA/RSA	175, 177
Atrazine	R-$SOCH_3$	Couple directly to sulfhydryl and lysine-NH_2 groups of haemocyanin	176, 178
Atraxine and simazine	At(6)-NH$(CH_2)_5$COOH	Raised in sheep	188

Cyanazine	R(4)-NH-$(CH_2)_3$-CONH Prot.		137, 180
Terbutryn	R-$SOCH_3$	Couple directly to sulfhydryl and lysine NH_2 groups of BSA	194, 318
Atrazine	R-Cl	"Derivatize and conjugate to BGG using a modified CDI procedure"	194, 318
Atrazine and hydroxyatrazine	R(4)-NH$(CH_2)_4$COOH	NHS Active Ester Path; BSA/KLH	196
Atrazine and Simazine	(a) R(4)NH$(CH_2)_n$COOH (b) R(2)S$(CH_2)_2$COOH	(a) Replace 4-Cl of cyanuric acid with amino acid; (b) replace 2-Cl of cyanuric acid with mercaptopropanoic acid; couple using NHS Active Ester Path; BSA/CONA/KLH/THY	198
Atrazine	D-NH$(CH_2)_6$COOH	Use CMC to link to KLH	28
Atrazine and Propazine	R-NH$(CH_2)_6$COOH	CDI using (a) CMC (b) DCC (c) EDC; BSA	183
s-Triazines	(a) $RSOCH_3$ (b) R-Cl	Couple directly to free NH_2 groups; BSA	197
Bentazon	R-N-$CH_2C_6H_4CH_2$COOH	NHS Active Ester Pathway; BSA/KLH	234
Imazamethabenz	R-COOH	Water-soluble CDI path using EDC; HSA	232
Imazaquin	D-CH_2HO	Couple to BSA or OA using hydrazide linkage	233
Amitrole	R-SH, -OR, -COOH	Library of 13 antigens prepared using 7 haptens and variety of spacer groups; hapten's NH_2 group was protected	231
Trifluralin	R-C_2H_4COOH	Synthesize propionic acid trifluralin; conjugate using Mixed Anhydride method	235
Norflurazon	R-C_4H_6COOH	Synthesize butyric acid derivative of 4-NH_2-Norflurazon; conjugate to BSA and RSA using Mixed Anhydride procedure	236
Deethylatrazine and deisopropylatrazine	R-$(CH_2)_5$COOH	Couple to BSA using CMC	185
Permethrin	Phenothrin-COOH	Mixed Anhydride method; KLH and BSA	240
Cypermethrin	PBA-COOH CYP-COOH	CDI method	137

TABLE 1 (continued)
Immunogen Preparation

Analyte	Reactive group	Coupling pathway	Ref.
Aldicarb	R-C(O)Cl	Convert to aldicarb oxime chloroformate; couple to *trans*-4-(amino-methyl)cyclohexane carboxyllic acid and then conjugate to BSA using Mixed Anhydride Path	268
Methoprene	RC_4COOH	Esterify with 4-hydroxy butanoic acid and couple to protein using NHS and HNSA Active Ester Paths; HSA	270, 271
Paraquat	R-COOH	Prepare hexanoic acid derivative of monoquat; couple to BSA/KLH using CDI	202, 204, 205, 208
Paraquat	R-COOH	Prepare valeric acid derivative of monoquat; couple by (a) Mixed Anhydride Path, or (b) activate using 1,2-dihydro 2-ethoxyquinoline-1-carboxylate and couple to BSA, KLH	203, 213
Paraquat	R-COOH	Prepare valeric acid derivative; convert to mixed anhydride using isobutyl chlorocarbonate; couple to KLH; OA; HSA; BSA; CONA	210, 211
Diclofop-methyl	D-COOH	Convert ester to acid; couple to NH_2 using CMC[d] (water-soluble CDI); BSA	214
Clomazone	Amino-Clomazone	Diazo linkage	215
Clomazone	p-NH_2-Clomazone	Diazo linkage; BSA	216
Alachlor	R-Cl	Thiolate proteins and couple directly to hapten	217
Metolachlor	Ar-$O(CH_2)_4COOH$	NHS Active Ester Method; BSA/KLH	221
Metolachlor, amidochlor, butachlor	R-Cl	Thioether linkage; HSA, IgG	218
Metazachlor	R-Cl	Thiolate proteins and couple directly to hapten	222
Maleic hydrazide	(a) R-COOH	(a) NHS Active Ester Path; KLH	223
Chlorsulfuron	D-C_6H_4-NH_2	Diazonium Path; KLH; BSA	225

Phenyl urea herbicides	(a)(Cl_{0-2})Ar-NH-C(O)N(CH_3)(CH_2)$_3$COOH (b)(CO_2H)(CH_2)$_2$C(O)NHArR	Couple to protein using EDC; HSA; Use mixed anydride path to prepare coating antigen	227
Triasulfuron	(a)Triasulfuron-NH(CH_2)$_3$OH (b)Chloroethoxy sulfonamide-(CH_2)$_2$COOH	(a)Diazonium path; problems with spacer rearrangement (b)NHS Active Ester Path; BSA; KLH	226
Methabenzthiazuron	R-NH_2	Diazo linkage to BSA	228
Molinate	(a)D-(CH_2)$_n$-COOH (b)D-C_6H_4-NH_2	(a) Mixed Anhydride Path (b) Diazonium Path; BSA/KLH/OA/THY/FIB/CONA	229
Aldrin (Dieldrin)	Analog-COOH	Active Ester Path; HSA	122
Chlordane		Derivatize at C-2 and couple to BGG using CDI	237
Endosulfan	D-(OH)$_2$	Convert diol to hemisuccinate using succinic anhydride; NHS Active Ester Path; KLH	238, 239
S-Bioallethrin	D-OH	Convert to ester using succinic anhydride; Active Ester Path; BSA/KLH	241
Diflubenzuron and BAY SIR	R-COOH	(a) NHS Active Ester Path; (b) Water-soluble carbodiimide path using CMC[d]; BSA; KLH; OVA	246, 247
Parathion	R-NH_2	Diazonium Path	86, 249
Parathion	(i) 2-Amino parathion (ii) Reduced parathion	Several strategies evaluated: (a) Diazo linkage; (b) couple (i) and (ii) using glutaric di-aldehyde; (c) convert (i) to amide using succinic anhydride and then use Mixed Anhydride Path; BSA; RSA	250
Paraoxon	R-NH_2	Diazonium Path; KLH/BSA	252, 254, 257
Paraoxon	(1) R-P-Cl (2) R-P-NHCH_2CH_2COOH	(1) Conjugate directly to BSA (2) NHS Active Ester Path; BSA	255
Paraoxon	R-NH_2	Succinyl spacer group	251
Soman	R-(C_6H_5)-NH_2	Diazonium Path; BSA	258, 259
MATP	NH_2	Diazonium Path	260, 261

TABLE 1 (continued)
Immunogen Preparation

Analyte	Reactive group	Coupling pathway	Ref.
Organophosphates	(a) DCP-COOH (b) TPB-COOH	Water soluble CDI using EDC and dioxane to improve solubility; BSA	256
Fenitrothion Chlorpyrifos-methyl Primiphos-methyl	Use an O-methyl-N-(tertbutylpropanoate)phosphoro-aminothioyl.Cl arm	Prepare appropriate succinimide ester and couple to protein	266
DDA	R-COOH	Convert to acid chloride using $SOCl_2$; couple to protein via amide bond; Bovine fibrinogen	121
DDA	R-COOH	React DDA anhydride with BSA/RSA	267
DDT	R-COOH	Convert to anhydride and conjugate to NH_2; BSA	120
Malathion	D-(CO_2)O	Couple the anhydride to BSA	120
Malathion	D-COOH	Convert to acid chloride using $SOCl_2$; couple to protein using amide bond; bovine fibrinogen	121
2-Aminobenzimidazole	R-NH_2	React with thiophosgene to form the isothiocyanate; couple directly to NH_2; OVA	123
Benomyl, methyl 2-benzimidazole-carbamate, thiabendazole	R-NH_2	Convert to amide using succinic anhydride; Couple to NH_2 using water soluble EDC[a] (Water soluble CDI Path); BSA	277, 278
Metalaxyl	R-COOH	Water-Soluble CDI Path; HSA	280
Triazole	R-OH	Convert to ester using succinic anydride; couple to BSA via Mixed Anhydride Path	286
Triadimefon	RCO; Reduce C=O to C-OH	Convert to ester using succinic anhydride; couple to HSA using Water-Soluble CDI	281
Iprodione	R-COOH	Water Soluble CDI Path; HSA	282
Fenpropimorph	R-COOH	NHS Active Ester Path; BSA	283

Ochratoxin A	R-COOH	Convert to mixed anhydride using isobutyl chlorocarbonate; couple to BSA	300
Triton-X detergents	OH	Convert to ester using succinic anhydride; Mixed Anhydride Path; KLH; BSA	290
Blasticidin S	BLS-NH_2	Acylate BLS using MBS; use sodium borohydride to reduce disulfide bonds in BSA; conjugate MBS-hapten to reduced protein	287
Surflan	-NH_2	Activate gel using NHS; couple to hapten's sulfonamide group; Affigel 10	313
Mercury	Glutathione-$HgCl_2$	CDI reaction; KLH	306

[a] 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide.
[b] N-hydroxysuccinimide.
[c] dicyclohexylcarbodiimide.
[d] 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-4-toluene sulfonate.

Note: Linkages are to NH_2 groups of the protein molecule, except for diazonium salts which are linked to tyrosine, histidine, tryptophan, and other residues.

A variety of methods that were originally developed for the conjugation of steroids, pharmaceuticals, and plant hormones to carrier proteins have been adapted for use with environmental contaminants (Table 1). Haptens that contain native or introduced carboxyl groups can be coupled via a mixed anhydride or acid anhydride directly to NH_2 groups on the protein.[26,29,30] The mixed anhydride reaction tends to minimize the formation of excessive cross-links in the protein molecule.[31] Haptens that contain carboxyl groups also can be conjugated using the straightforward water-soluble carbodiimide (CDI) method,[4,16,26] or the CDI-based N-hydroxysuccinimide (NHS) activated ester reaction.[26,32] The latter method also minimizes cross-linkage reactions because the activated ester can be separated from the CDI reagent and stored for future use. Water-soluble CDI methods are most suited to water-soluble or unstable haptens.[4] Haptens that contain aromatic NH_2 groups can be diazotized and coupled directly to proteins.[16,33] Hapten NH_2 groups also can be derivatized using an acid anhydride, or an acid chloride of a dicarboxyllic acid and then coupled to protein via the remaining -COOH group. Haptens that contain free amine or sulfhydryl groups also can be conjugated using CDI methods,[34] although this approach has not been commonly used for environmentally important analytes. Hydroxyl groups can be converted to the half ester using an acid anhydride and subsequently coupled using one of the conjugation methods developed for carboxyl groups, such as the mixed anhydride method.[35] There are a variety of conjugation methods available for use with other functionalities.[24,34]

2. Antisera

Once purified and characterized, the immunogen is used to induce the production of ABs in a suitable host animal. Purification of the immunogen helps to narrow the range of affinities and specificities of the induced PABs. Because the titer, specificity, and avidity of sera tend to vary from animal to animal, the chances of a satisfactory immune response are increased if several animals are immunized. PABs are most often raised in rabbits, which are readily available, inexpensive to husband, and have responsive immune systems. The host animal is injected intramuscularly or via multiple intradermal routes with an emulsion of the immunogen in a suitable adjuvant.[25,27,36] The adjuvant, usually Freund's, slows the immunogen's release from the injection site and stimulates the immune response.[4] When the AB titer is satisfactory, blood is collected from the central ear vein or by cardiac puncture. After clotting, the serum is separated from the blood cells by centrifugation and stored at –70°C.

If necessary, ABs that recognize the immunogen's linkage arm can be removed by affinity chromatography. Either the AG or the linkage arm is immobilized on a support bed; the matching ABs are removed by passing the serum through the column.[29,36,37] Sera are selected for use in IAs on the basis of their avidity, which can be estimated by means of an antiserum displacement curve, and specificity for the target compound.[38]

3. Monoclonal Antibodies (MABs)

Although most assay formats are not unduly influenced by the AB's clonal type,[27,39] several of the shortcomings of polyclonal sera, such as finite production, can be overcome by means of hybridoma technology. MABs have stable specificity and affinity and are produced by segregated and immortalized lines of lymphocyte B cells.[40,41] Most commonly, spleen lymphocytes from immunized mice are isolated and then fused with myeloma cells. The resultant hybridomas are purified, cloned, and screened for the ability to produce high-affinity ABs of the desired specificity. The selected clones are used to produce ABs either in cell culture or in the ascites fluid of mice where the MABs are formed in high yields. Detailed descriptions of MAB production techniques are available from other sources.[13,16,40-42] The clonal screening process can be used to enhance an assay's specificity; clones can be selected that are not overly influenced by the hapten-protein linker arm and are specific for the target molecule. MAB-based assays are noted for high specificity toward their target molecules, a feature that arises from the ability of most MABs to recognize a single antigenic determinant. The steep dose-response curves of many MAB-based assays contributes to assay precision.[4] Moreover, because of the clonal screening process, the immunogen

does not need to be highly pure.[4,27] Hybridoma technology is not problem free, however. MABs are costly to produce;[4,16,27,42] the chromosome complement of the hybrid cells can be unstable;[4,39] and assay specificity can be too narrow for some screening tasks.

4. Immunoassay Types

The main types of immunoassay are characterized by the type of tracer used to quantify the analyte. In most IA variants, a decrease in the tracer's activity relative to an analyte free control is inversely proportional to the analyte's concentration. Radioisotopes and enzymes are the most commonly used labels; however, fluorescent and chemiluminescent labels are gaining popularity. Depending on the assay format, the label is incorporated into either the ABs (primary or secondary) or the hapten.

a. Radioimmunoassay (RIA)

In RIAs (Figure 3), which are competitive-binding IAs, the reagents are usually adjusted so that 30 to 50% of the tracer is bound in the absence of analyte. After equilibrium binding is achieved, the bound label (usually) is quantified. Analyte levels in unknown samples are interpolated from an assay calibration curve (Figure 3).[38] Radioligands, especially those that emit gamma radiation, can be rapidly, conveniently, and sensitively counted.[35,43,44] Picogram level determinations are usually possible using RIA.[6] Unlike some nonisotopic IAs, the RIA's final quantification step is not affected by nonspecific interferences.[31,45]

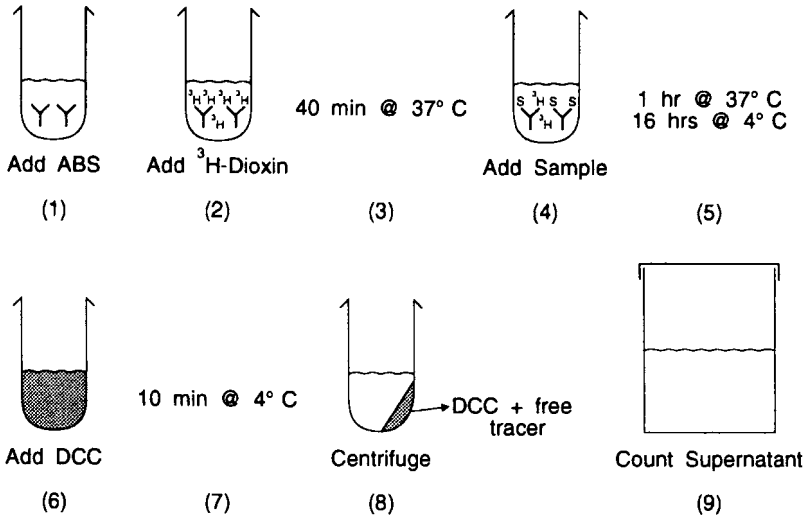
^{125}I, which combines high isotopic abundance with high specific radioactivity (2170 Ci/mmol),[6] is the most commonly used isotope. As a gamma emitter, ^{125}I is easily and efficiently counted without the need for scintillation fluids.[4,31,45] Tritium or ^{14}C-labeled ligands (specific activity: 29.2 Ci/mmol and 62.4 mCi/mmol, respectively,[46]) have lower specific activities and require the use of a liquid scintillant.[5,7] For the latter two tracers, a greater mass of radioligand is required to match the counting efficiency of an iodinated radioligand: the result is decreased assay sensitivity. The most sensitive RIAs are based on ^{125}I-labeled radioligands.[44,47]

Ideally the ABs should have similar affinity for the radioligand and analyte.[25] One advantage of tritiated ligands, which are often available commercially,[8] is that they usually bear a closer resemblance to the unlabeled hapten than do iodinated ligands. In addition, the longer half life of [^3H] (12.5 years) reduces the need for the regular synthesis of fresh batches of tracer. Tritiated ligands are widely used in IAs for steroids and drugs mainly because these compounds can be difficult to iodinate without affecting their antigenicity.[6,48] Tritium-based assays tend to be less sensitive than their iodinated counterparts,[7,38] although Weiler et al.[27] point out that the differences can be slight.

To minimize steric hinderance by the large iodine atom, the hapten can be derivatized with an easily labeled phenolic or imidazole group.[49,50] Tyramine or histamine units, which can be separated from the parent molecule by a spacer arm, are common choices;[51] the subunits may be labeled before or after conjugation to the hapten.[5,45,52] The spacer arm can affect assay performance, however. If the radioligand's spacer arm too closely resembles the immunogen's, the tracer may be more avidly bound than the analyte, which will reduce assay sensitivity.[53,54] This occurs if the serum contains ABs to the immunogen's linkage arm or its site of attachment to the hapten. Bridge recognition is one of the chief disadvantages of radioiodinated tracers.[54] ^{125}I-based assays that use homologous bridges are often less sensitive than their [^3H] counterparts.[54] Heterologous linkages that differ in either structure or site of attachment to the hapten can reduce the effect of bridge recognition.[43,50,54] ^{125}I is substituted into the hapten by chemical (e.g., Chloramine-T) or enzymatic (e.g., lacto-peroxidase) driven reactions. In these reactions, $Na^{125}I$ is oxidized; cationic ^{125}I is then rapidly incorporated into the phenolic or imidazole residue.[5,6,31,45] Radioligands of high specific activity are essential for sensitive RIAs; the incorporation of one iodine atom per molecule is considered desirable.[50]

i. Separation Systems

With the exception of scintillation proximity assays,[55] the reliability of RIAs depends on the physical separation of the bound and free radiotracer fractions. Phase separation procedures should be efficient, simple, inexpensive, and should not



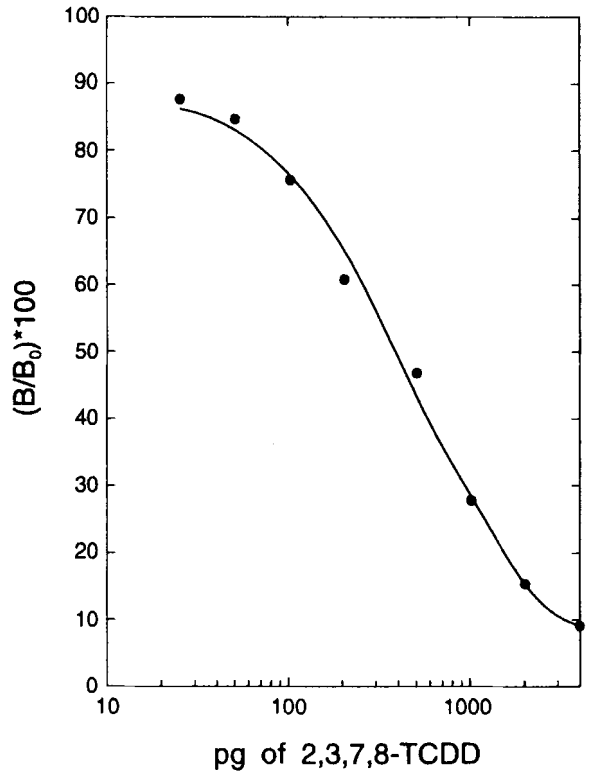


FIGURE 3. Tritium-based RIA for PCDDs; assay outline and typical calibration curve.

disturb the equilibrium between the binder and ligand.[6,56,57] Most of the popular separation methods exploit either physicochemical or immunological principles.

The physicochemical methods, which include fractional precipitation by salts or solvents,[25,31] are simple, fast, cheap, and reproducible but tend to have high assay blanks (5 to 20%),[46,56] which can affect assay performance at trace analyte levels.[5,6] Such blank effects can be reduced by washing the precipitate.[38] Adsorption of free tracer by dextran-coated charcoal (DCC) is widely used in hapten assays.[31,58] DCC methods are convenient, inexpensive, and well suited to large sets of assay tubes.[5]

The double antibody (DAB) separation technique, which is probably the most popular of the

immunological separation methods, exploits the spatial separation of the ABs antigenic and binding sites.[31] Antibodies raised against the primary host's IgG are used to precipitate the AB-ligand complex. DAB methods are reproducible, nondisruptive,[38,43,59,60] and usually have low assay blanks (1 to 3%).[5,59]

Either the primary or secondary ABs can be immobilized on a solid phase such as polypropylene tubes[5,31,51] or particles of latex, sepharose, or sephadex.[5] Immobilization of the ABs on magnetizable particles can eliminate the need for centrifugation.[35,46] Solid-phase methods are versatile, have low nonspecific adsorption effects,[31,46] and have fewer error sources than other methods.[60] On the debit side, particulate solid-phase reagents are expensive and immobilization may reduce the serum's avidity.[5,31]

There are sound theoretical reasons why noncompetitive assays, in which the number of AB binding sites that are occupied by analyte molecules are quantified, have the potential to be far more sensitive than competitive binding assays such as RIA, which use labeled analytes to quantify the number of AB binding sites that are unoccupied by analyte molecules.[44,47,61] Theoretically, the sensitivity of a noncompetitive labeled AB assay could reach a detection limit of a single molecule if a sufficiently active tracer and detection system were available;[44,62] in practice, their sensitivity is limited by the label's specific activity.[19,44,47] For these reasons, the noncompetitive immunoradiometric assay (IRMA) format, which uses labeled ABs in excess quantities,[19] is unlikely to prove more sensitive than conventional RIA methods unless a nonisotopic label of higher specific activity is used.[19] IRMA is not suited to small haptens since it requires two well-separated epitopes per target molecule — one site is bound by the capture ABs and the other by the labeled AB.

Scintillation proximity assays use DABs that are coated onto microspheres containing a fluorophore to bind the primary ABs. Radioactive decay emissions from the bound tracer excite the fluorophore and the emitted light is quantified in a scintillation counter.

b. Enzyme-Immunoassay

Concern about the health effects of low-level radiation and strict regulations governing the use of radioisotopes were the stimuli for interest in alternative IA labels. Enzyme immunoassays (EIAs), which were introduced by Engvall and Perlmann[63] and van Weeman and Schuurs,[64] resemble RIA except that enzyme activity rather than radioactivity is measured. EIAs overcome some of RIA's limitations[9] through improved safety, superior versatility,[65] and longer lived reagents.[31,66] Moreover, EIAs can be easily adapted for use in the field.[69] The EIA and RIA formats are comparably sensitive[67] and cost effective. Some ultrasensitive EIAs that are noncompetitive in design and use fluorometric techniques to measure the enzyme's activity can detect antigens at a levels of 10^{-18} to 10^{-21} mol — which is far lower than can be achieved by RIA.[68] In these assays, the use of fluorometry improved the sensitivity of enzyme detection by 1000 times. Nonspecific binding (NSB) of the tracer, which can erode assay sensitivity, was drastically reduced by immobilization of the ABs on very small polystyrene spheres.

On the other hand, enzymatic end-points are more difficult to determine with precision than gamma-emitting labels and they require an extra assay step.[66] Slight variations in reaction conditions can affect enzyme activity,[16] although the differences in precision between state-of-the-art EIAs and RIAs are now considered marginal.[31,67] Enzyme-based assays, particularly homogeneous EIAs, may be more susceptible to interferences than are the RIAs.[66]

In the present review, the term EIA is used to describe all IAs that depend on enzyme-labeled reagents for the signal quantification step.[66,67] A multiplicity of EIA formats have been developed. The characteristics of the main EIA variants are briefly described in the next section. The application at hand and the nature of the sample matrix usually determine which format is preferable. Most commonly, ABs or coating AG are adsorbed on a solid phase to facilitate phase separation,[16,70] usually a 96-well plastic microtiter plate is used for this purpose. The microtiter plate is an imperfect support, and alternative solid phases are receiving attention (see Reference 71 for a review). The coated plates are stable for 3 to 6 months.[72] The optical density (O.D.) of the developed plates are read in microtiter plate readers, which are available from commercial sources. The levels of the various reagents are selected by means of checkerboard titrations and trial calibration curves.[16,42]

i. Enzyme Tracers

The most popular enzyme labels are horseradish peroxidase, glucose oxidase, alkaline phosphatase, and β-galactosidase.[16,31,67,69,73] A variety of reagents such as glutaraldehyde and sodium periodate are used to conjugate the enzyme labels to ABs.[69] The methods listed in Table 1 can be used to conjugate enzymes to haptens.[16,67,69,72] As with RIAs, bridge recognition by the ABs can be reduced by means of a heterologous assay design or affinity-purified serum.[27]

ii. EIA Formats

EIAs can be divided into two main categories: homogeneous and heterogeneous assays. These categories can be further subdivided into competitive and noncompetitive (immunometric) assays.[70] Unlike homogeneous assays, heterogeneous assays require separation of the bound and free phases before the enzymatic end-point is determined. The following section is intended to introduce the reader to the more popular EIA formats. The reader is referred to other publications for a more comprehensive overview of the many EIA variants.[62,74]

Homogeneous EIAs — In *competitive homogeneous EIA* [enzyme-multiplied IA technique (EMIT)] the activity of the enzyme, which is conjugated to the hapten, is modulated by the binder.[66,67] EMIT assays are efficient and precise because the number of assay steps is minimized.[31] Since the mechanism of enzyme inhibition is steric, EMIT assays are most suited to small AGs or haptens whose AB binding sites are close to the conjugated enzyme.[76] EMIT assays are extensively used in clinical chemistry[76] where they have tended to be less sensitive than their heterogeneous counterparts;[69,77] they are also susceptible to matrix interferences.[76]

Noncompetitive homogeneous EIAs are not very common; they use two enzyme-labeled MABs that are directed against different epitopes on the AG. One of the enzyme labels produces the substrate for the other. Detectable product is formed only when the enzymes are in close proximity;[16] that happens when the MABs are bound to AG.

Heterogeneous EIAs — *Competitive heterogeneous EIAs* are the equivalent of conventional RIAs[31,67] and are the most popular format for residue tests.[77,78] They are often referred to as *enzyme-linked immunosorbent assays* (ELISA) because either the ABs or AG is immobilized on a solid phase. In the *direct competitive EIA* (Figure 4), the AB is adsorbed on the solid phase. Immobilized AB as-

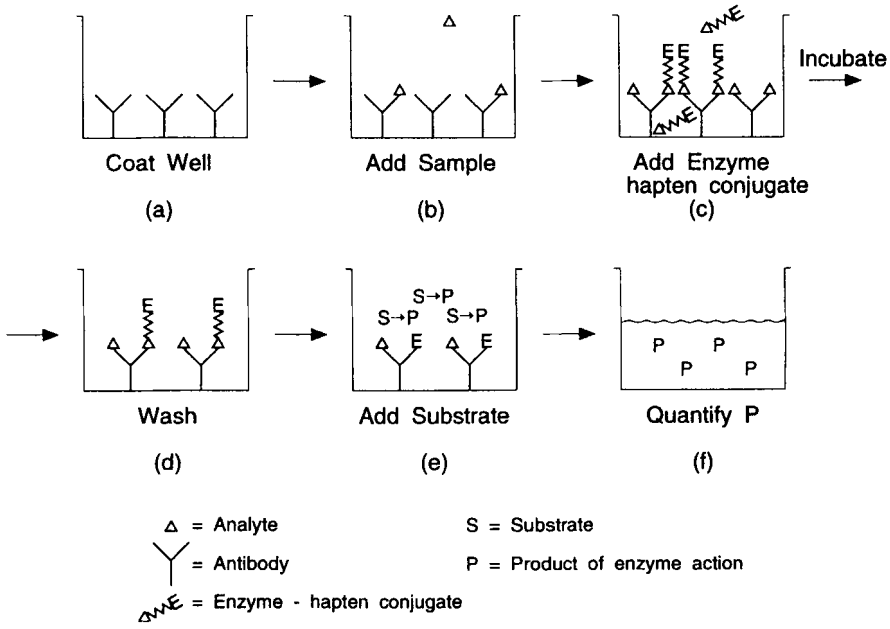


FIGURE 4. Direct competitive enzyme immunoassay.

says require the co-incubation of sample and enzyme tracer, which can expose the tracer to harmful matrix components.[79] After the main incubation step, the unbound reagents are washed from the microtiter wells and the amount of enzyme activity that has been bound by the ABs is measured. A decrease in enzyme activity is proportional to the amount of analyte present. A variant of the direct competitive heterogeneous EIA uses labeled ABs. In that format, a calibrated amount of protein-hapten conjugate is immobilized on the solid phase; the latter reagent, the sample, and a limited amount of enzyme-labeled ABs are then incubated together. After a phase separation step, the amount of primary antibody that has bound to the adsorbed hapten is used to deduce the analyte's concentration.[10]

In *indirect competitive EIAs* (Figure 5), the AG is adsorbed on the solid phase and, after a competitive binding step, an enzyme-labeled double AB is used to measure the amount of primary AB that has bound to the coating AG. The use of enzyme-labeled DABs, which are available commercially, prevents possibly harmful matrix components from coming in contact with the enzyme label.[10]

Noncompetitive heterogeneous EIAs (enzyme-immunometric assays) are equivalent to IRMA except that an enzyme tracer replaces the ABs' isotopic label. This format uses an excess of labeled antibody and is capable of low detection limits. Although the acronym ELISA is often loosely applied to all solid-phase IAs that use enzyme-labeled reagents,[66] it also can be used more restrictively to describe noncompetitive solid-phase heterogeneous assays.[69] ELISAs are commonly used to detect ABs[16] and are excess reagent assays in which the amount of bound enzyme label is directly proportional to the analyte's concentration.[69] To be suitable for detection in such a sandwich assay, an analyte must have multiple AB binding sites.[66] Noncompetitive assays often have enhanced specificity because two sites on the analyte must be bound by the reagent ABs.

c. Fluorescent and Chemiluminescent Labels

Fluorescent labels such as fluorescein, rhodamine, and the rare earth chelates have emerged as promising nonisotopic labels.[31,80,81] The labeling techniques are relatively simple and the tracers have an indefinite shelf life.[80] The quantification of fluorescent labels rivals gamma counting for rapidity

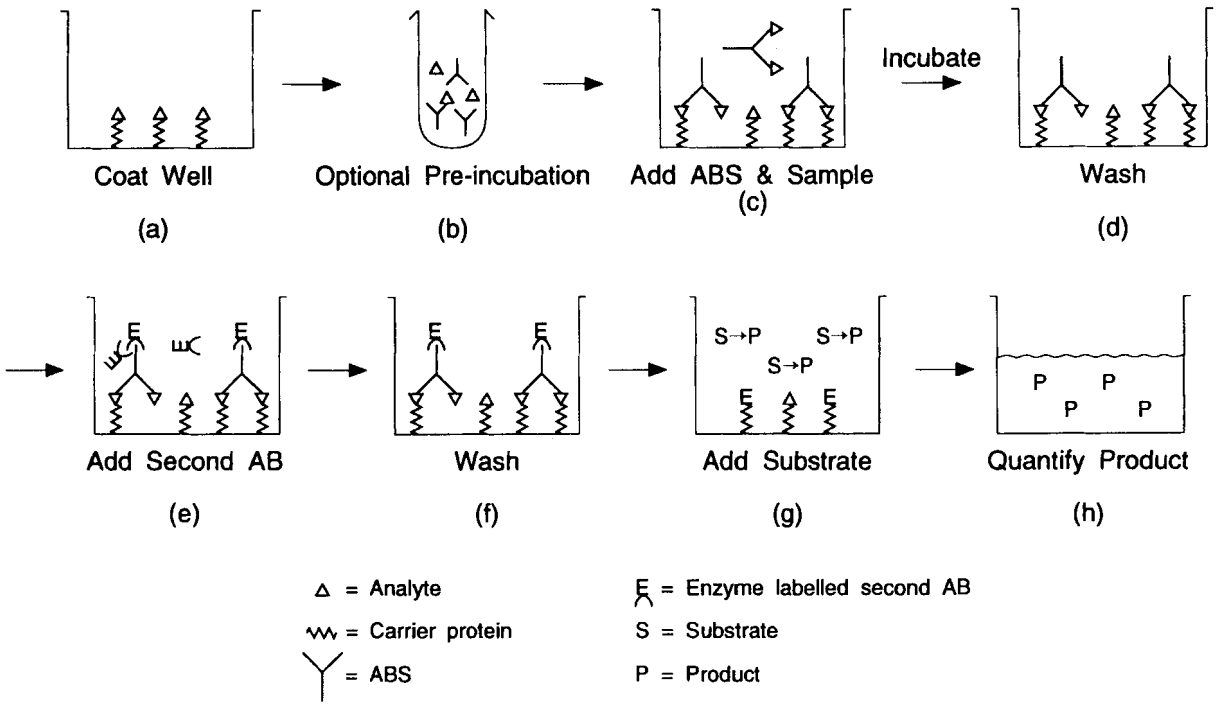


FIGURE 5. Indirect competitive enzyme immunoassay.

and precision.[80] Fluoroimmunoassays (FIAs) have been developed in competitive and noncompetitive formats. The use of the chelated rare earth fluorophores should help improve the sensitivity of the FIA technique by reducing the effects of background signals, which was a problem in earlier FIAs.[81] The use of fluorescent labels of high specific activity also should facilitate an improvement in the sensitivity of noncompetitive, labeled AB-type assays below the limits imposed by radioisotope labels.[44, 82]

Chemiluminescent labels such as luminol can be used as tracers in chemiluminescence immunoassays (CIA) that in several cases have proven to be as sensitive as RIA or EIA systems.[83] The luminol label is oxidized in the presence of H_2O_2 and a catalyst such as microperoxidase.[84] Wider use of the CIA technique has been impeded by inefficient detectors and the tendency of sample components to interfere with signal detection.

5. Advantages and Limitations of IA Techniques

There is general consensus that the many advantages of the IA technique outweigh its limitations (Table 2). Nevertheless, it is important to recognize that IAs have definable limitations — as do most other techniques. One of IA's most serious drawbacks, and this factor perhaps more than any other has hindered the wider use of environmental IAs, is the difficulty and cost of developing a fully fledged and certified IA. The many steps in the assay development process are outlined in Figure 6. The early stages of the process that involve the design and synthesis of a suitable hapten are the most critical and difficult. This work requires creative input from analytical and synthetic chemists and from immunologists. Inasmuch as it is unrealistic to expect individual laboratories to develop their own haptens and sera, it is essential that antisera be widely distributed and readily available. This has yet to happen on a wide scale, although a few private sector companies are striving to improve matters (see Section VII).

IAs differ in several important ways from conventional analytical techniques. Since ABs can cross-react with compounds that closely resemble the target analyte, IAs can produce false-positive results when used to analyze complex environmental

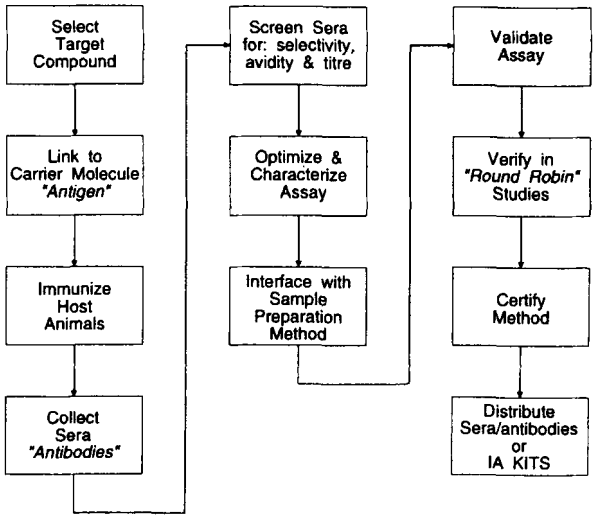


FIGURE 6. Steps in the development of an environmental immunoassay.

samples. That limitation can be advantageous if the intent is to detect classes of related compounds. On the other hand, it means that IAs are not quantitative techniques in the absolute sense. Positive samples should be independently confirmed if a high level of confidence is to be maintained. Confirmation of all samples may be unnecessary in some monitoring applications if the IA has been carefully validated for the system under observation. IAs also are totally unsuited to the detection of unknown compounds in environmental samples. IAs can be deceptively easy to use; the interests of accuracy and precision are best served if the semiquantitative formats are undertaken by analysts who are technically skilled and scientifically aware.

Although IAs are unsuitable for small sample sets, their efficiency and cost effectiveness improve as the sample load increases. IAs have generally low detection limits (Tables 3, 4, and 5) that compare well with those of the best conventional methods. In the case of polar analytes, low DLs (detection limits) can often be achieved with little or no sample preparation. Usually, 10 ml of water is sufficient for an immunoanalysis, which is orders of magnitude less than is required for many conventional methods. Trace analysis of polar analytes can often be achieved by the inclusion of a simple concentration step. Moreover, most EIA formats permit the simultaneous analysis of 30 to 40 samples. The detection of trace levels of hydrophobic analytes such as the halogenated hydrocarbons presents special problems. If low DLs are to be reached for those

TABLE 2
Advantages and Limitations of Immunoassay Techniques

Advantages	Ref.	Limitations	Ref.
Wide applicability	4, 22, 37, 77, 307	High development costs	9, 22, 244, 307
Complement GC or LC methods	2, 22, 37, 39, 85, 210	Haptens can be difficult to synthesize	78
Sensitive	4, 8, 9, 22, 38, 39, 87, 123, 307	Limited availability of antisera	70, 77, 78, 86
Specific	4–6, 39, 85, 123, 134	Can be vulnerable to cross-reacting compounds and non-specific interferences	6, 8, 24, 78, 386
Cost-effective analysis of small-volume samples	6, 8, 39, 70, 78, 85, 88	Requires independent confirmation	17, 78, 340
Rapid with high sample throughput	6, 8, 9, 38, 39, 65, 70, 78, 242	Inappropriate for small sample loads or multi-residue determinations	6, 78, 85, 130
Ease of use	70, 72	Lack of acceptance, conservative attitudes	9, 77, 85
Usually reduced sample preparation	6, 39, 70, 72, 127, 210, 242, 244, 307		
Simultaneous analysis of multiple samples	39		
Ideal for large sample loads; easily automated	2, 6, 8, 39, 77, 78, 85, 86, 88, 127, 130		
Suited to field use	2, 17, 37, 85		

TABLE 3
Immunoassays for Trace Organic Contaminants

Analyte	ABs	Format	Label	Sep. sys.	Range (dl) buffer	Range (dl) matrix	Ref.
PCDDs 2,3,7,8-TCDD	P	RIA	1-N-[5-^{125}I valeramido] 3,7,8-TCDD	DAB[a]	F12[b] cutscum: 50 pg-75 ng (25 pg); I_{50}: 20 ng F12 triton: 20 pg–2 ng; I_{50}: 1 ng GC5 cutscum: 200 pg–20 ng; I_{50}: 4 ng	Adipose tissue: (100 pg/60 mg) (1.67 ppb)	29, 144
PCDDs 2,3,7,8-TCDD	M	RIA	^{125}I-labeled second AB	SP	Inadequate competition		151
PCDDs	P	RIA	1-N-[5-^{125}I valeramido] 3,7,8-TCDD	DAB	12.5 pg-1 ng (27 pg)	Fish: (67 ppt)	146, 147
PCDDs 2,3,7,8-TCDD	M	EIA	Enzyme labeled second AB	IH[c]	100 pg–10 ng (<500 pg) I_{50}: 1–2.5 ng		152, 153
PCDDs	M	EIA	Enzyme labeled second AB	IH	40 pg–10 ng (0.1 ng) I_{50}: 1 ng (0.25% Cutscum); 5 ng (1% Cutscum)	Chemical samples: (1 ppb; 1 ng)	130, 155
PCDDs 2,3,7,8-TCDD	p	RIA	^{3}H-labeled 2,3,7,8-TCDD (40 Ci/mmol)	DCC[e]	(a) 20 pg–2 ng (21 pg) I_{50}: 350 pg (b) 2.5 pg–200 pg (3.9 pg); I_{50}: 42 pg		150
PCDFs	P	RIA	4-N-[5-^{125}I valeramido] 2,7,8-TCDF	DAB	20 pg–4 ng (20 pg) I_{50}: 150 pg		124
PCBs 4-MCBP 453′4′TCBP 262′6′TCB	P	RIA	^{125}I-labeled iodovaleramido BP	DAB	2-NH_2-3′,4′,4,5-TCBP: 0.5–100 ng (0.5ng)[d], I_{50}: 5 ng; (dl-3,4,3′,4′-TCBP/2,6,2′,6′TCBP: 1.0ng)	25-450 ng Aroclor 1254; 5–300 ng other BPs and Aroclors	125
PCBs 22′44′55′HCBP	P	RIA	2-[^{125}I iodo] 2′,4,4′,5,5′-HCBP	DCC	Aroclor 1260: 0.1-3 ng (100 pg); I_{50}: 0.4 ng	Milk: 20–80 ppb (20 ppb); blood: 2–16 ppb (2 ppb)	160
PCBs Aroclor 1248		EIA			(8.4 ng/well)	17 ppb soil extract (concentrated 50×)	161
PCP	M	Probe	Capacitative affinity sensor				370
PCP	M	EIA	Enzyme labeled second AB	IH	10 ng/ml–1 μg/ml (30 ng/ml); I_{50}: 68 ng/ml	Water: (84 ppb); SPE: (25 ppb); Solvent extraction: (1 ppb)	166

4-acetamidobiphenyl	P	RIA	^{125}I-labeled-tyramine derivative (16 Ci/mmol)	DAB	66 pg–40 ng (5.7 pg) I_{50}: 1 ng		162
N,N′-diacetylbenzidine	P	RIA	^{125}I-labeled-tyramine derivative	DAB	8.3 pg–530 pg (6.2 pg) I_{50}: 100 pg		163
Benzo-a-pyrene	M	EIA	Enzyme labeled second AB	IH	3×10^2 fmol–10^4 fmol (3×10^2 fmol)[2]; I_{50}: 4 pmol	Urine: I_{50}: 12-15 pmol	164
2,4,6-TNT		EIA	Labeled hapten	IAB	20 pg/ml–20ng/ml (20 pg/ml) I_{50}: 350 pg/ml	Tap water: 0.05–10 ppb	167
4-nitrophenol & mono subs. 4-NPs	P	EIA	Labeled DAB	IH	0.3 ng/ml–140 ng/ml (2nM)[d] I_{50}: 8 ng/ml	Water: (0.2 ppb)	169

[a] Double AB method.
[b] Serum pool.
[c] Immobilized hapten.
[d] Estimated from lower limit of calibration curve.
[e] Dextran coated charcoal.

TABLE 4
Immunoassays for Herbicides

Analyte	ABs	Format	Label	Sep. sys.	Range (dl) buffer	Range (dl) matrix	Ref.
(1) 2,4-D and (2) 2,4,5-T	P	RIA	^{125}I-labeled hydroxyl phenyl diazene derivative;	PA[a]	0.1–100 ng (200 pg)[b] (1) I_{50}: 6.4 ng/100 µl (2) I_{50}: 1.4 ng/100 µl	(1) (100 ppt) (river water)	170
2,4-D	P	RIA	(a) ^{125}I-labeled tyramine derivative (b) Tritiated 2,4-D (12.6 Ci/mmol)	PEG	(a) Did not compete (b) I_{50}: 4.5 p*M*	In urine: 0.1–100 ng (100pg); I_{50}: 1 ng	172,173
2,4-D	P	EIA	Labeled haptens: (a) b-galactosidase conjugated through -COOH moiety; (b) b-galactosidase conjugated to 4-position on aromatic ring	IA	(a) 5–500 ng (10 ng); I_{50}: 50 ng/100 µl (b) 5–500 ng (50 ng); I_{50}: 500 ng/100 µl	In water: (5 ppb)	171
2,4-D	P	EIA; RIA	(a) Labeled DAB (b) [^{3}H]glycine-hapten	IH $(NH_4)^bSO_4$	(a) 100 ng/ml–10 µg/ml (100 ng/ml)[b]; I_{50}: 2 µg/ml (b) 50 ng/ml–10 µg/ml (50 ng/ml)[b]; I_{50}: 1 µg/ml	In river water and urine: (a) 100 ppb–10 ppm (b) 50ppb–10 ppm	174
Triazines (Atrazine)	P	EIA; RIA	(a) Biotinylated DAB/streptavidin peroxidase (b) ^{125}I-labeled tyrosine derivative	IH	(100 pg/ml) (1 ng)		175,177
Atrazine	P	EIA	Labeled hapten	IAB[c]	IgG: 1.1–2200 ng/ml (1.1 ng/ml); I_{50}: 10.8 ng/0.2 ml. Affinity purified ABs: 11 pg/ml -33 ng/ml (11 pg/ml); I_{50}: 108 pg/0.2ml.		176,178
				IAs[d]ph	IgG: 11 pg/ml–55 ng/ml (11 pg/ml); I_{50}: 21.5 ng/20 ml. Affinity purified ABs: 110 fg/ml–550 pg/ml (100 fg/ml); I_{50}: 108 pg/20 ml		
Atrazine	P	EIA	Labeled hapten		(20 pg/ml)		184

Terbutryn	P	EIA; RIA	(a) enzyme labeled DAB IAsph (b) [^{14}C]Terbutryn	IAB 1.9 ng –14.4 µg (1.9 ng)[b] SP	4.8–290 ng/assay (4.8 ng); I_{50}: 69 ng/assay Similar to EIA	River water: 25–1500 ppb (25 ppb) 0.1–600 ppb (0.1 ppb)	181,182
Cyanazine	P	EIA		IH		(0.5 ppb in water; 0.005 ppb with SPE; 0.01 ppm in soil)	137,180
Atrazine	P	EIA	Labeled hapten	Tu[e] IAB	0.5–10 ng/ml (0.1 ng/ml) (50 pg–1 ng/tube); I_{50}: 0.4 ng/ml 30 pg/ml–5 ng/ml (20 pg/ml); I_{50}: 250 pg/ml	Whole milk: 0.2–6.4 ng/ml (0.2 ng/ml)	194; Ferguson, personal communication; 318
Atrazine/simazine 188	P	FIA	Labeled hapten	IAB	0.01–1.0 ng/ml		
(1) Atrazine and (2)-hydroxy atrazine	M	EIA	Labeled DAB	IH	(1) 50 pg/ml–5 ng/ml (50 pg-/ml); I_{50}: 450 pg/ml (2) 50 pg/ml–20 ng/ml (50 pg/ml): I_{50}: 500 pg/ml	Water (50 ppt)	196
Triazines	P	EIA	Labeled DAB	IH	Simazine: 1.1–3400 ng/ml; I_{50} 110 ng/ml Atrazine: 0.2–100 ng/ml; I_{50}: 10 ng/ml	Water (0.3 ppb–1 ppm)	198,199

TABLE 4 (continued)
Immunoassays for Herbicides

Analyte	ABs	Format	Label	Sep. sys.	Range (dl) buffer	Range (dl) matrix	Ref.
(a) Deethyltrazine (b) Deisopropylatrazine	P	EIA	Labeled hapten	IAB	(a) 0.01 ng/ml–100 ng/ml (10 pg/ml; I_{50}: 0.2 ng/ml (b) 0.01 ng/ml–100 ng/ml (10 pg/ml); I_{50}: 0.28 ng/ml		185
Atrazine/propazine	P	EIA	Labeled hapten	IAB	Atrazine: 0.1–1000 pg/ml (1 pg/ml; I_{50}: 20 pg/ml Propazine: 0.1–1000 pg/ml (0.1 pg/ml): I_{50}; 10 pg/ml		183
s-Triazines	M	EIA	Labeled hapten	IAB	Terbutryn: 0.1–10 ng/ml (0.1 ng/ml); I_{50}: 0.4 ng/ml Prometryn: 0.3–10 ng/ml (0.3 ng/ml); I_{50}: 1.1 ng/ml Aziprotryn: 1–100 ng/ml (1 ng/ml); I_{50}: 4 ng/ml		197
Atrazine	P	EIA	Labeled DAB	IH	0.01–10 ng/ml (0.03 ng/ml) I_{50}: 0.2 ng/ml		28
Atrazine	M	EIA	Labeled hapten	IDAB	0.01–10 ng/ml (0.03 ng/ml) I_{50}: 0.25 ng/ml200		200
(a) Atrazine (b) deethylated atrazine		EIA	Labeled hapten	IAB	(a) 0.005–10 ng/ml (5 pg/ml) (b) 0.02–10 ng/ml (20 pg/ml)	(a)Soil: 1 μg/kg (b)Soil: 2 μg/kg	186
Terbuthylazine	M	FIA	Labeled hapten	IAB	(a) 0.01–10 ng/ml (56.5 pg/ml); I_{50}: 0.3 ng/ml		191,192
Terbuthylazine	M	EIA	Labeled hapten	IAB	(a) 0.01–10 ng/ml I_{50}: 0.3 ng/ml		193
Amitrole	P	EIA	Labeled DAB	IH	(0.02 μ*M*–50 μ*M* (0.02 μ*M*)		231
Paraquat	P	RIA			50–1600 ng/ml (50 ng/ml); I_{50}: 100 ng/ml		201

Paraquat	P	RIA		C[f]	0.5–10 ng (0.6 ng/ml: Niewola et al.[205] cite a DL of 6 ng/ml for the RIA) Sensitivity of optimized assay = 120 pg/ml)	In serum: (0.6 ng/tube)	202
Paraquat	P	RIA	(a) [^{3}H]Paraquat (1.35 Ci/mmol)	SP[g]	(a) 1–100 ng/ml (0.8 ng/ml); I_{50}: 10 ng/ml	(a) In serum: 10–250 ppb (3.6 ppb) (b) (0.25 ppb in serum)	203
			(b) ^{125}I-labeled phenol derivative (130 Ci/mmol)	SP[g]	(b) 0.29–28.6 ng/ml (0.1 ng/ml)		
Paraquat	P	EIA	Labeled DAB	IH	(0.3–10 ng/ml); I_{50}: $8x10^{-7}M$		204
Paraquat	M; P	EIA	Labeled DAB	IH	(M): 0.8–12 ng/ml (0.5 ng/ml);	In serum: 0.8–50	205, 206, 311
					I_{50}: 3 ng/ml	ppb (0.5 ppb); In soil (200 ppb)	
					(P): 0.08–10 ng/ml (0.08 ng/ml)[b]; I_{50}: 2 ng/ml		
Paraquat	P	EIA	Labeled DAB	IH	0.1–27 ng/ml; I_{50}: 2 ng/ml	Variety of matrices: (0.1–>100 ppb); milk: (<1ppb) I_{50}: 0.3 ppb	211, 212
Paraquat	M	EIA	Biotinylated DAB	IH	1–10 ng/well (0.3 ng/well); 10–100 ng/ml (3 ng/ml); I_{50}: 1.1 ng/well (11 ng/ml)		208
Paraquat	P	FIA	Fluorescein-paraquat	DAB	20–2000 ng/ml (20 ng/ml) I_{50}: 100 ng/ml		213
Diclofop-methyl	P	EIA; FIA	(a) Enzyme-labeled hapten	DAB+PEG	(a) 10–75 ng/ml (23 ng/ml); I_{50}: 27 ng/ml	(a) soil: (0.023 ppm); milk: (0.23 ppm); urine: (0.115 ppm)	214
			(b) Fluorescein-labeled hapten	PEG	(b) 20–200 ng/ml (45 ng/ml; 9 ng/tube); I_{50}: 32 ng/ml	(b) soil: (0.045 ppm); milk: (0.45 ppm); urine: (0.225 ppm)	
Clomazone	P	EIA	Labeled DAB	IH	0.5–500 ng/ml (0.5 ng/ml)[b]; I_{50}: 12 ng/ml	Soil extracts: 0.01–10 ppm	215
Clomazone	P	EIA	Labeled DAB	IH	1.37-250 ng/ml (1ng/ml); I_{50}: 25 ng/ml	Soil extracts (5 ppb)	216

TABLE 4 (continued)
Immunoassays for Herbicides

Analyte	ABs	Format	Label	Sep. sys.	Range (dl) buffer	Range (dl) matrix	Ref.
Alachlor	P	EIA	Labeled DAB	IH	Deionized H_2O: 0.2–8 ng/ml (0.2 ng/ml)[b]; I_{50}: 0.5 ng/ml		217
Alachlor	P	EIA	Labeled hapten	IAB	0.1–10 ng/ml (50 pg/ml) I_{50}: 1 ng/ml	Water: 0.5–10 ng/ml	219
Metolachlor	M	EIA	(1) Labeled hapten (2) Labeled DAB	(1) IAB (2) IH	(1) 0.1–10 ng/ml (0.1 ng/ml) I_{50}: 1 ng/ml (2) 0.05–10 ng/ml (0.05 ng/ml) I_{50}: 0.6 ng/ml		221
(a)Metolachlor (b)Amidochlor (c)Butachlor	P	EIA	Labeled DAB	IH	(a)I_{50}: 6 ng/ml (b)I_{50}: 2 ng/ml and 0.2 ng/ml (c)I_{50}: 7 ng/ml		218
Metazachlor	P	EIA	Labeled hapten	IABs	10–1000 pg/ml (10 pg/ml) I_{50}: 100 pg/ml		222
Maleic hydrazide	M	EIA	Labeled DAB	IH	10 ng/ml–11 μg/ml (0.11 μg/ml); I_{50}: 0.84 ppm	(1 ppm in potato extract)	223, 224
Chlorsulfuron	P	EIA	Labeled DAB	IH	I_{50}: 90 ng/ml	In soil: (0.4 –1.2 ppb)	225
Triasulfuron	M	EIA	Labeled DAB	IH	0.01–1 ng/ml (10 pg/ml) I_{50}: 0.05 ng/ml	Soil: 0.1–10 μg/kg (0.1 μg/kg)	226
Methabenzthia-zuron	P	EIA	Labeled hapten	IAB	50 pg/ml–10 ng/ml (50 pg/ml); I_{50}: 1 ng/ml		228
Urea (Carbamide) herbicides	P	EIA	Labeled DAB	IH	(a) Monolinuron 0.08–5 ng/ml; I_{50}: 0.9 ng/ml (b) Diuron 0.08–5 ng/ml; I_{50}: 1.6 ng/ml (c) Linuron 0.5–50 ng/ml; I_{50}: 12 ng/ml	(a)Potato (22 ng/g), wheat (14 ng/g) (b) Potato, wheat (0.14 μg/g)	227
Imazaquin	P	EIA	Labeled hapten	PA-JAB	0.45–25 ng/ml; I_{50}: 10.7 ng/ml		233

Imazamethabenz	P	EIA	Labeled DAB	IH	0.5–32 ng/ml I_{50}: 12.6 ng/ml	12.5–200 ppb in wheat and barley	232
Bentazon	P	EIA	Labeled DAB	IH	I_{50}: 110 μM		234
Molinate	P	EIA	Labeled DAB	IH	3–3000 ng/ml (3 ng/ml); I_{50}: 80 ng/ml	Performance in water same as in buffer (practical limit 15 ng/ml)	229
Molinate	P	EIA	Labeled DAB	IH	10–500 ng/ml (21 ng/ml); I_{50}: 106 ng/ml		78, 331
Molinate	P	EIA	Labeled DAB	IDAB	I_{50}: 20 ng/ml		78
Molinate	P	EIA	Labeled DAB	IH	(15 ng/ml)	(10 ppb in water; 0.1 ppb in water extract; 30–60 ppb in soil extract)	39, 320
Picloram	P	RIA	[^{3}H]glycine-hapten	$(NH_4)_2SO_4$	50–5000 ng/ml (50 ng/ml); I_{50}: 760 ng/ml	River water and urine: 50–5000 ppb	174
Picloram	P and M	EIA	Labeled DAB	IH	(P): 5–5000 ng/ml (5 ng/ml); I_{50}: 140 ng/ml (M): 1–200 ng/ml (1 ng/ml); I_{50}: 10 ng/ml	Affected by matrix interferences Water: 20–2000 ppb; soil 4–400 ppb	230
Thiobencarb	P	EIA	Labeled DAB	IH	20–1000 ng/ml (1 ng/ml; practical 10 ng/ml); I_{50}: 100 ng/ml		39
Trifluralin	P	EIA	Labeled DAB	IH	(0.1–1 ppm)		235
Norfluazon	P	EIA	Labeled DAB	IH	1–10000 ng/ml (1 ng/ml); I_{50}: 250 ng/ml		236

[a] Protein A.
[b] Estimated from lower limit of calibration curve by present author.
[c] AB immobilized on microtitre plates.
[d] ABs immobilized on polystyrene beads.
[e] ABs immobilized on tube walls.
[f] Charcoal.
[g] Solid phase AB.

TABLE 5
Immunoassays for Insecticides

Analyte	ABs	Format	Label	Sep. sys.	Range (dl) buffer	Range (dl) matrix	Ref.
(1) Aldrin and (2) Dieldrin	P	RIA	[^{125}I] tyramine derivative (3.3 Ci/mmol)	DAB	(1) 2–100 pmol (700 pg); I_{50}: 8 ng (2) 0.2–100 pmol (150 pg); I_{50}: 1.6 ng		122
Chlordane	P	EIA	Labeled hapten	IH	2.5–80 ng/tube (25 ng/ml);[a] I_{50}: 110 ng/ml	Soil extracts: 0.8–3000 ppm	237
Endosulfan	P	EIA	Labeled hapten	IAB	3–400 ng/ml (3 ng/ml);[a] I_{50}: 135 ng/ml		238
s-Bioallethrin	P	RIA	[^{3}H] tyramine derivative	C	0.1–10 nmol (0.1 nmol); I_{50}: 1.2 nmol		242
	P	EIA	Labeled DAB	IH	10-100,000 ng/ml (2ng/ml); I_{50}: 1 µg/ml 0.0016–1.6 nmole ; I_{50}: 10 ng/ml	Milk: I_{50}: 1 ppm	210, 243
(1) Diflubenzuron and (2) BAY SIR 8514	P	EIA	Labeled DAB	IH	(1) 8–200 ng/ml (8 ng/ml);[a] I_{50}: 48 ng/ml (2) 10–400 ng/ml (10 ng/ml); I_{50}: 120 ng/ml	Water: (1 ppb) Milk: (40 ppb)	247
			Labeled primary AB	IH	(1) 2–200 ng/ml; I_{50}: 15 ng/ml (2) 2–200 ng/ml; I_{50}: 37 ng/ml		
(1) Diflubenzuron, (2) BAY SIR 8514, and (3) Penfluron	P	EIA	Labeled DAB	IH	(1) 0.5–15 ng/ml (0.5 ng/ml); I_{50}: 3 ng/ml (2) I_{50}: 9 ng/ml (<1 ng/ml); (3) I_{50}: 6.8 ng/ml (<1 ng/ml)	Milk: (2 ppb) 10 ppt with clean-up	248
Organophosphates	P	EIA	Labeled DAB	IH	I_{50}: 10^{-6}–10^{-4} mol/l ($I_{50} \times 5^{-1}$)		256
Parathion	P	RIA	^{3}H (300 mCi/mmol) or ^{14}C (40 mCi/mmol) labeled hapten	DCC	4–400 ng (4 ng); I_{50}: 50 ng	Plasma and lettuce (10–20 ng) ≡ 0.1 ppm	249
Parathion	P	EIA	Labeled DAB	IH	(5–10 ng/ml); I_{50}: 58 ng/ml	0.025–0.05 ppm (fruit, veg., serum extracts)	386

Paraoxon	P	EIA	Labeled DAB	IH $8 \times 10^{-5} M$	$10^{-10} M$–$10^{-3} M$ (28 pg/ml); I_{50}:	Serum: (280 pg/ml)	252, 253
Paraoxon	M	EIA	Labeled DAB	IH	10–100 μg/ml (1 μg/ml); I_{50}: $7 \times 10^{-5} M$		254
Paraoxon	P	RIA	[^{3}H]		100 pg–3.2 ng (200 pg) I_{50}: 800 pg		333
Paraoxon	P	EIA	Labeled hapten	PT[b]	($10^{-6} M$)	Water: ($10^{-6} M$)	257
Paraoxon	P	(1) RIA	[^{3}H]paraoxon	(1)$(NH_4)_2SO_4$	(1): 0.7–70 pmols (300 pg) I_{50}: 7 pmols		255
		(2) EIA	[^{3}H]AChH	(2) homogenous	(2): 10^{-7}–$10^{-6} M$ ($10^{-7} M$)		
Paraoxon	P	RIA	^{125}I-labeled hapten				251
Soman	M	EIA	Labeled DAB	IH	$10^{-6} M$–$10^{-3} M$ (200 ng/ml); I_{50}: $8 \times 10^{-3} M$		258
MATP (Soman-/OP Analog)	P	EIA	Labeled DAB	IH	10^{-8}–10^{-4} mol/l (10^{-10} mol/l) I_{50}: 10^{-6}mol/l		260
MATP (Soman-/OP Analog)	M	EIA	Labeled hapten	IAB	10^{-7}–10^{-4} mol/l (1.3×10^{-7} mol/l; 975 pg/assay or 39 ng/ml)	Various matrices: (2.1×10^{-7}–4.9×10^{-8} mol/l)	261
MATP	M	CIA			Field assay (10^{-6} mol/l) Luminometer (5×10^{-8} mol/l)	Water, serum, milk: (10^{-6} mol/l)	263
Soman	M	EIA	Labeled hapten	IAB	10^{-7}-10^{-4} mol/l (5×10^{-7} mol/l; 100 ng/ml)	Various matrices: (1.3×10^{-6}–2.0×10^{-6} mol/l)	262
Cypermethrin (PBA)	M	EIA			(50 ppb)		137
Permethrin	M	EIA	Labeled DAB	IH	0.4 ng–20 ng (0.4 ng);[a] I_{50}: 1.55 ng	50 -500 ppb in beef	240
DDA	P	EIA	Labeled DAB	IH	10–100 ng/ml (10 ng/ml);[a] I_{50}: 72 ng/ml	Urine: ng level	267
Aldicarb	P	EIA	Labeled hapten	IAB	15.6 ng–2 μg (300 ng/ml) I_{50}: 125 ng	0.3 ppm stream water, 0.6 ppm plasma	268, 269
Aldicarb	P	EIA	Labeled hapten	IAB	1–100 ng/ml (250 pg/ml); I_{50}: 27 ng/ml		220
(a) Fenitrothion	M	EIA	Labeled hapten	IAB	(a)1–1000 ng/ml (0.3 ng); I_{50}:	Grain:	266

TABLE 5 (continued)
Immunoassays for Insecticides

Analyte	ABs	Format	Label	Sep. sys.	Range (dl) buffer	Range (dl) matrix	Ref.
(b) Chlorpyrifosmethyl (c) Primiphosmethyl	M P				28ng/ml (b)0.8–1000 ng/ml (0.2 ng); I_{50}: 29 ng/ml (c)0.5–100 ng/ml (0.02 ng); I_{50}: 2 ng/ml	(a) (0.08 ppm) (b) (0.2 ppm) (c) (0.03 ppm)	
Methoprene	P	EIA	Labeled hapten	(a)IAB (b)IH	0.1–100 ng/ml (250 pg/ml); I_{50}: 3 ng/ml 5–30 ng/ml (10 ng/ml);[a] I_{50}: 50 ng/ml	Tobacco: 1–10 ppm Wheat extract: (60 ppb)I_{50}: 750 ppb	270, 271
Bacillus thuringiensis sub sp. *kurstaki* toxin	P	EIA	Labeled DAB	IH	0.03–3 μg/ml (0.03 μg/ml);[a] I_{50}: 6 μg/ml		272
Bacillus thuringiensis sub sp. *kurstaki* toxin	P	EIA	Labeled toxin	IAB	20–1000 ng/ml (20 ng/ml);[a] I_{50}: 105 ng/ml		273
Bacillus thuringiensis sub sp. *israelensis* toxin	P	EIA	Labeled DAB	IH	15–1000 ng/ml (15 ng/ml);[a] I_{50}: 200 ng/ml	Water: no clean-up (100–1000 ppb); with SPE step (100 pg/ml)	274

[a] Estimated from lower limit of calibration curve by present author.
[b] Pinch test format.

analytes, the extracts of oily matrices must be enriched so as to remove residual lipid materials — and even then, femtogram-level detection limits are not yet possible. The inclusion of clean-up steps detracts from the usefulness of IAs as a screening technique. The immunoanalysis of lipophilic analytes is discussed in the present report.

Since IAs are inherently adaptable, the creative analyst can often adjust the assay format or manipulate assay parameters so as to overcome perceived deficiencies.[85] Some more advantages of IA technology are listed in Table 2 and will be explained by example in the following sections. From many perspectives, IAs are a most promising screening technique.[2,8,85-88] At its present state of development, the technology is not yet suited to multiresidue applications, although there are signs that sensitive multianalyte IAs will emerge in the next decade.

II. ENVIRONMENTAL APPLICATIONS

A. Screening Tests

Many of our current and future environmental problems are a direct result of modern society's release of a myriad of man-made chemicals into the environment. This situation is hardly surprising since about 500,000 chemicals are in use worldwide,[89] and about 76,000 of those chemicals are in daily use.[90] The chemical emissions of industry, agriculture, and municipalities often end up in surface or ground water.[91,92] Water is a major transportation vector for chemical pollutants; the oceans and large inland lakes are the ultimate sinks.[93] The hundreds of new compounds that enter the market place annually will aggravate the crisis unless remedial action is taken.[91,94]

In addition to their concern about the environmental damage that chemical pollutants cause, the public is worried about possible health effects — even though much of the evidence that links environmental pollutants to health problems has yet to be quantified, remains controversial,[91,92,95-97] and is often misunderstood.[98] A common response to such concerns is to regulate the use and emission of problem chemicals. Exposure and consumption guidelines are usually based on worst-case estimates of adverse health effects. The intent is to minimize exposure to harmful agents as part of the effort to improve public health.[99] Concerns have been raised about the scientific validity of regulatory policies that are based solely on the foregoing philosophy without an adequate regard for the vast array of natural toxins to which humans are exposed.[98]

The best way to conserve and protect the aquatic environment is to control pollution at its source. The extent of pollution and the effects of control and remedial measures on affected ecosystems also must be assessed. The surveillance and monitoring of aquatic environments for chemical contaminants can be an onerous and costly task. Inasmuch as humans are exposed to aquatic pollutants mainly by the consumption of contaminated water or biota,[100] much attention is focused on those matrices. Analytes that require enrichment and the use of sophisticated detectors can be particularly difficult to determine.[1,101-103] Many laboratories are barely able to cope with current sample loads, and those loads are likely to increase in response to regulatory and political pressures. Clearly, there is a need for new and improved analytical methods to help boost productivity and lower costs.[104] At present, it is common for laboratory staff to spend much time processing samples that are either analyte-free or contain negligible analyte levels. Methods that could screen out these "negative" samples, preferably after minimal sample preparation, would lower the cost of many analyses.

There are two main screening strategies: one is to detect biological or biochemical effects that are induced by contaminants; the other is to selectively detect target contaminants. The former approach includes a wide variety of short-term mutagenicity and toxicity tests. The Ames test, which is probably the best known mutagenicity test, uses the induced reversion of mutant genes in strains of *Salmonella typhimurium* to estimate a sample's mutagenic and carcinogenic potential.[89,105,106] Since many carcinogens are mutagenic in *in vitro* tests,[105,107,108] the short-term mutagenicity tests can be used to rank samples according to carcinogenic potential and potential health risk.[109] There are many other short-term mutagenicity tests,[109,110] most of which can be readily adapted to environmental tasks. Mutagenicity[111] and cytogenicity tests[112,113] have been used to detect the effects of chemical pollution in aquatic environments. In the absence of an all-purpose method for the assessment of environmental

health,[114] batteries of short-term biochemical, [115] genotoxicity, and bioassay tests[116] have been used to screen samples.[90] Positive samples can be fractionated and reanalyzed so as to identify the toxicant.[117,118] Unfortunately, many carcinogens test negative in the various short-term mutagenicity tests,[107] and genotoxicity tests, although they do tend to detect the more potent carcinogens, do not detect carcinogens such as benzene and many of the halogenated aromatic hydrocarbons.[107,108,119]

IAs are among the most promising techniques for the detection of targeted contaminants. Historically, metabolites of DDT [1,1,1-trichloro-di-(4-chlorophenyl)ethane] and malathion were the first molecules used to induce the formation of reagent antibodies to environmental contaminants.[120,121] Centeno et al.[120] suggested that their anti-DDA and anti-malathion ABs could be used with radio- and fluorescein labels to locate pesticide residues in plant tissue. Ercegovich extended that concept in a pivotal article that proposed the use of immunological screening methods for the rapid detection of pesticide residues.[86] He foresaw that IAs could be used not only to rapidly screen large sample sets, but also to confirm the results of conventional analyses. An RIA for the insecticides aldrin and dieldrin was the first reported assay for an environmental contaminant.[122] An IA for 2-aminobenzimidazole, a degradation product of the fungicide Benomyl,[123] and a set of assays for some important chlorinated contaminants soon followed.[29,124,125]

Inasmuch as IAs are ideal for determining the absence of analyte, they can expedite the elimination of "negative" samples from large sample sets. A strategy for the inclusion of an IA screening step in an analytical protocol is illustrated in Figure 7. Since IAs are not intended to replace conventional techniques, all positive samples and a random subset of negative samples should be confirmed by an independent technique. As confidence and experience grow, the number of negative samples that must be confirmed can be reduced. Residue chemists have been encouraged to explore the cost-saving potential of IAs by the International Union of Pure and Applied Chemistry (IUPAC) Commission on Pesticide Chemistry.[87] The screening out of "negative samples" would be particularly beneficial in the case of trace contaminants that require complex clean-up procedures and sophisticated instrumental quantification.[39,126] IA screening techniques

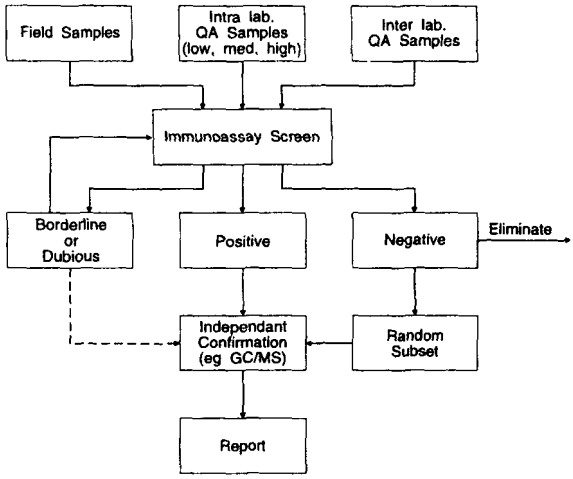


FIGURE 7. Immunoassay screening strategy.

can lower average analytical costs by a factor of five,[2] and for many analytes could eliminate the need for extensive sample clean-up.[87] It now costs about $1 billion a year to monitor environmental contaminants in the U.S.[2] Clearly, there are considerable savings to be made. The maturing interest in environmental IAs is evident from the reviews[4,37,39,77,78,85,88,127-133,391] and supportive articles[11,22,134-138] that have been published in the past decade. The commercial prospects for environmental IA kits also have been assessed.[2,128,129]

The following section is an overview of IAs that have been developed for the major classes of environmental contaminants: trace contaminants, herbicides, insecticides, and fungicides. Assay performance characteristics, assay validation, and sample preparation are considered in subsequent sections.

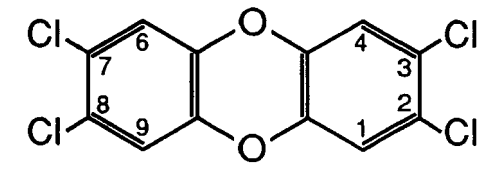
B. Halogenated Aromatic Hydrocarbons (HAHs) and Related Compounds

Several groups of halogenated aromatic compounds are notorious as trace contaminants, mainly because of their toxicity toward test animals, persistence in the environment, and tendency to accumulate in the food chain. Public and scientific concern has created an acute need for data that describe the distribution, occurrence, and fate of organohalogen contaminants in the environment. HAHs tend to be difficult to analyze because they are lipophilic and often have multiple congeners. In the mid-1970s,

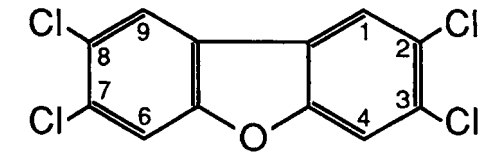
researchers at the National Institute of Health Sciences in the U.S., recognizing the potential of IAs as a screening tool, developed RIAs for the following HAH pollutants: polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-*p*-dioxins (PCDDs) (Table 1). That pioneering research demonstrated the ability of IA screening techniques to detect members of this important class of environmental contaminants. In the process, some serious problems related to the poor solubility of HAHs and the development of suitable radioligands were overcome.

1. PCDDs/PCDFs

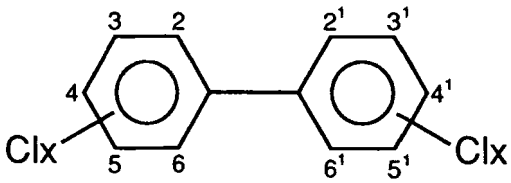
The 75 PCDD congeners, commonly referred to as "dioxins," have caused more concern than almost any other organic pollutant. 2,3,7,8-T_4CDD (Figure 8), the most notorious dioxin congener, is extremely toxic to some mammals such as the guinea pig; other 2,3,7,8-substituted congeners are also very toxic. Although some PCDDs are considered to be potent carcinogens in test animals,[107] there is continued controversy about the nature and magnitude of the "dioxin threat" to human health. Kimbrough states that, based on present data and known exposure levels, the PCDDs may not be a serious health hazard for the general population.[139] Recent evidence, however, indicates that some PCDDs, PCDFs, and co-planar PCBs not only cause a variety of effects at the subcellular level, many of which are manifested clinically in animals and humans,[140,141] but also are apparently carcinogenic in humans where they may act as long-term promoters of a variety of cancers.[142,143] Unfortunately, many of the key epidemiological studies have been confounded by exposures to other potentially carcinogenic chemicals.[143] PCDDs are lipophilic and recalcitrant compounds that tend to bioaccumulate in the food chain. They are formed as byproducts during the manufacture of chlorophenols and phenoxy herbicides, and during the chlorine-assisted removal of lignin from pulp products. PCDDs also are formed during the combustion of many materials in the presence of chlorine. Conventional methods for the determination of PCDDs, which couple extensive clean-up with high-resolution gas chromatography and mass spectrometry, are time consuming and expensive ($1000 to $2000 per sample).



2,3,7,8-tetrachlorodibenzo-p-dioxin



2,3,7,8-tetrachlorodibenzofuran



Polychlorinated biphenyls

FIGURE 8. Molecular structures of PCDDs, PCDFs, and PCBs.

Albro et al. developed an RIA for PCDDs in an effort to reduce the number of samples that must be analyzed by mass spectrometry.[29] Because AB-AG binding is an aqueous phase reaction, hydrophobic analytes such as PCDDs should be solubilized in the assay buffer.[144] The solubilization system should render the ligands accessible to the ABs without unduly interfering with the binder-ligand reaction. The nonionic surfactants Cutscum and Triton X-305 (0.5%) were the most effective of 15 detergents that Albro's group tested for the ability to solubilize 2,3,7,8-T_4CDD. The Triton-based assay was the more sensitive, probably because Cutscum inhibits AB binding (15 to 20%) more than Triton (1 to 2%).[125] The Cutscum-based assay, however, had a wider working range and greater capacity. The antisera, which were raised in rabbits, were screened

for selectivity, low recognition of the immunogen's adipamide group (Table 1), and high affinity for the dioxin portion of the radioligand.[145]

The RIA was highly specific for PCDDs and PCDFs: of several related compounds that were tested, only the co-planar 3,4,3′,4′-TCBP cross reacted (*CR% = 6). The two selected sera, F-12 and GC-5, differed in their ability to bind a spectrum of PCDD and PCDF congeners. GC-5 had greater avidity than F-12 for several P_5- and H_6-substituted PCDD congeners, making it more suitable for screening applications. The authors speculated that a radioligand with higher specific activity (Table 3) or a more avid serum would help improve the assay's sensitivity.[144]

Dimethyl sulfoxide (DMSO) also proved useful as a solubilization agent in the RIA for PCDDs: a DMSO-based assay outperformed Cutscum, Triton, and horse serum solubilization systems at low 2,3,7,8-T_4CDD levels.[146] The DMSO-based assay's working range was from about 20 pg to 2 ng and its calibration curve was the steepest of the four assays. Reduction of the assay's incubation time from 72 to 24 h did not adversely affect assay performance. When fortified Trout extracts were analyzed, however, DMSO was found to be more prone to matrix overload effects than Triton.[147]

The radioligand used in the foregoing versions of the RIA for PCDDs (^{125}I iodovaleramido-PCDD) had several shortcomings that adversely affected assay performance and reliability. The unlabeled hapten was unstable; it tended to lose iodine during storage and to cyclize, which resulted in variable yields from the labeling reaction. Also, the specific activity of the radioligand could not be readily increased by an enrichment step. Collier et al.[148] synthesized and characterized a tyramine derivative of 1-NH_2-3,7,8-T_3CDD that had similar solubility to 2,3,7,8-T_4CDD; it was hoped that this hapten would be easier to label in high yields. An anilide analog of the molecule was labeled with cold iodine using a biphase reaction that was driven by chloramine-T.[149] For unknown reasons, the tyramine-TCDD conjugate, however, has proven difficult to label with ^{125}I (unpublished data, present author). The availability of high activity (40 Ci/mmol) preparations of [^{3}H]2,3,7,8-T_4CDD (ChemSyn, Lexana, TX) prompted an investigation into its use as an alternative radioligand in the RIA.[150] A sensitive [^{3}H]2,3,7,8-T_4CDD-based RIA was developed that could detect between 20 pg and 2.0 ng of 2,3,7,8-T_4CDD (Figure 3). The assay used DCC to separate the bound and free phases (Table 3).[150] The intra-assay precision of the [^{3}H]-based assay was apparently better than that of the ^{125}I-based assay's [coefficient of variation (CV) of <15% vs 20%]. A "low end" version of the assay that used reduced tracer (1500 cpm) and antiserum levels had a working range of 2.5 to 200 pg 2,3,7,8-T_4CDD.

The ability of hybridoma technology to improve the selectivity of the IAs for PCDDs has been explored. Kennel et al. used a novel solid-phase RIA to screen hybridoma cultures for the production of anti-2-NH_2-3,7,8-T_3CDD MABs.[151] The MABs were screened for the ability to bind immobilized BSA-T_3CDD. Although some of the MABs had high binding affinity for BSA-T_3CDD, they were unable to detect free 2,3,7,8-T_4CDD. Stanker and co-workers generated five MABs that were each capable of binding free 2,3,7,8-T_4CDD.[152,153] Their success was attributed to the use of free 2,3,7,8-T_4CDD in the clonal screening protocol. The MABs were used in a competitive indirect EIA. The analyte was solubilized in phosphate buffered saline (PBS) that contained BSA (1 mg/ml). The five MABs differed in their ability to recognize PCDD/PCDF congeners other than 2,3,7,8-T_4CDD; all, however, favored congeners with intermediate levels of chlorination.[154] None of the MABs recognized unchlorinated, mono-, hexa-, or octa-chlorodibenzo-*p*-dioxin. The production of MABs with good affinity for the hexa-octa chlorinated PCDDs/PCDFs would broaden the assay's applicability. Since the selected MABs recognized the immunogen's -NH_2 group as chlorine, the assay was more sensitive to 1,2,3,7,8-P_5CDD and 1,3,7,8-T_4CDD than to 2,3,7,8-T_4CDD. With the exception of the co-planar 3,3′,4,4′-T_4CBP, PCBs were not recognized. A later version of the EIA used Cutscum as the solubilization agent; assay performance was optimal at Cutscum levels of 0.125 and 0.25% (v/v).[155]

A sensitive RIA that could be used to detect 2,3,7,8-T_4CDF (Figure 8) in commercial preparations of PCBs as well as in environmental samples also has been reported.[124] PCDFs are present as contaminants in PCB preparations, and also are formed in large quantities when PCBs are burned. The assay had a working range of 20 pg to 4.0 ng 2,3,7,8-T_4CDF and was fairly specific for T_4CDF, although some cross-reactivity with similar compounds such as 2,3,8-T_3CDF, 2,3,6,8-T_4CDF, and 2,3,7,8-T_4BrDF was observed. Again, the hapten's

* CR, cross-reactivity.

amino group was recognized by the immune system as a chlorine atom. The RIA for 2,3,7,8-T_4CDF warrants evaluation with environmental matrices. The assay would be particularly useful for screening samples from the vicinity of PCB fires, which are known to generate large quantities of 2,3,7,8-T_4CDF.

2. PCBs

PCBs (Figure 8) are widespread environmental contaminants that are known to cause a variety of acute and chronic toxic effects in test animals and humans.[156-159] The Aroclors are commercially prepared PCB mixtures that are used as coolants in electrical transformers and capacitors. Aroclors differ in their chlorine content and in the average number of chlorine atoms per PCB molecule.[158,159] The need to detect Aroclors in food, biota, and the environment stimulated interest in an IA for PCBs. Antisera raised against the lower chlorinated PCBs should be more sensitive to Aroclors, such as 1242, that consist of predominantly lower chlorinated isomers. Luster et al. prepared antisera to several PCB derivatives (Table 1) for use in an RIA.[125] The sera had greatest avidity for their corresponding haptens and were fairly specific for the matching isomers. The serum raised against 2-NH_2 -4,5,3',4'-TCBP appeared to recognize the hapten's NH_2 group as a Cl atom since it was most sensitive to 2,4,5,3',4'-PCBP. Similarly the serum raised against 3-NH_2 -2,6,2',6'-TCBP proved more sensitive to 2,3,6,2',3',6'-HCBP (CR% = 43) than 2,6,2',6'-TCBP (CR% = 27.3). Small quantities of detergent were used to solubilize the hydrophobic analyte and radioligand. As was the case with the RIA for PCDDs, a Triton X-305 (0.5%)-based assay was more sensitive than its Cutscum equivalent, though Cutscum had a greater capacity. As anticipated, the antiserum to 4-NH_2-4'-MCBP was most sensitive to Aroclor 1242 and poorly responsive to higher chlorinated Aroclors; the antisera to 2-NH_2-4,5,3',4'-T_4CBP and 3-NH_2-2,6,2',6'-T_4CBP were most sensitive to the higher chlorinated Aroclors. A typical calibration curve for Aroclor 1254 had a shallow slope; about 400 ng of analyte caused a 15% assay response. The assay's sensitivity needs improvement before it can be used in most routine applications; that would probably require a more avid serum.

Subsequently, Newsome and Shields exploited the tendency of the immune system to recognize -NH_2 groups as Cl atoms by raising an antiserum to 2-NH_2-2',4,4',5,5'-P_5CB (Table 1).[160] The antiserum was expected to be more sensitive to the higher chlorinated PCBs that are common in Aroclor 1254 and 1260. Unlike the lower chlorinated formulations, the 1260 formulation is still considered to be carcinogenic in rats.[159] A radioligand with high specific activity (2073 Ci/mmol) was prepared by the addition of ^{125}I directly to 2-NH_2-2',4,4',5,5'-P_5CB via a Sandmeyer reaction. The tracer's proximity did not seem to unduly hinder antibody binding. DMSO (25%), which was used as a solubilization agent, permitted better AB-ligand binding than Cutscum. Furthermore, the inclusion of DMSO in the DCC phase separation reagent improved assay sensitivity tenfold, presumably by improving the free ligand's access to the charcoal adsorbent. The assay was most specific for the target congener. The calibration curves for Aroclor 1260 and 1254 were sensitive and steeply sloped; whereas, the lower chlorinated Aroclor 1242 produced about 90% less binding. Researchers at ECOCHEM (Minnesota) and Immunosystems Inc. (Maine) recently reported the development of an EIA that is highly sensitive for Aroclor 1248.[161]

Johnson et al.[162,163] reported RIAs for 4-acetaminobiphenyl and N,N'-diacetylbenzidine, which are metabolites of the carcinogens 4-aminobiphenyl and benzidine, respectively (Table 1). The radioligands were prepared by coupling tyramine to hemisuccinyl derivatives of the haptens by means of an NHS-based active ester reaction. A lactoperoxidase method was used to label the ligand with ^{125}I. A similar strategy could probably be used to label other chlorinated hydrocarbons such as PCBs and PCDDs. A MAB-based EIA has been used to detect benzo-a-pyrene (BP) and its metabolites in urine (Table 1).[164] The MABs cross-reacted with a broad range of BP metabolites and other polycyclic aromatic hydrocarbons (PAHs). Such broad specificity enhances the assay's usefulness as a broad screen for PAHs and their metabolites. The possibility of using these MABs to detect metabolites of 2-nitrofluorene in urine samples is currently being explored.[165]

Westinghouse Bio-Analytic Systems developed an indirect EIA for the wood preservative pentachlorophenol (PCP).[166] The MABs used in the assay were fairly specific for PCP: 2,3,5,6-T_4CP

cross-reacted 42% and 2,4,6-T_3CP cross-reacted 12%. Solubilization of the analyte was aided by the inclusion of isopropanol (25%) in the reaction mixture.

A direct competitive EIA has been developed for the determination of the explosive 2,4,6-trinitrotoluene and related nitroaromatic compounds in water samples (Table 3).[167] Variations in the length of the labeled hapten's spacer arm affected the assay's performance.[168] In the absence of a spacer, the ABs failed to bind the tracer. Variations in the length of the spacer arm from C3 to C6 caused the assay curve to shift to the right hand side. The very sensitive assay had a DL of about 20 pg/ml (Table 3). The ABs cross-reacted with 2,4-dinitrotoluene (DNT), 2,4-dinitroaniline, 1,3-dinitrobenzene, and 2,6-DNT. The cross-reactivities of these compounds increased sharply with the concentrations of the analytes.

Nitrophenols are important environmental contaminants both in their own right and as degradation products of some organophosphate pesticides.[169] It may even be possible to use nitrophenols to infer prior exposure to, or contamination with, the parent pesticide. With that objective, Li et al.[169] developed an indirect EIA for the determination of 4-nitrophenol and monosubstituted 4-nitrophenols. Three immunogens were used to immunize a total of eight rabbits. After extensive screening, AB1812 was selected for use in an optimized assay (Table 1). Similar rigorous screening was used to select an optimal coating AG for the assay. The assay was pH sensitive — possibly because the nitrophenols are ionizable. The assay's performance improved considerably when the concentration of the incubation buffer was increased. Some monosubstituted nitrophenols reacted strongly in the assay and had similar I_{50} values, which means that the assay should be suitable for the detection of this group of compounds.

C. Herbicides

1. Phenoxy Acid Herbicides

The widely used chlorinated phenoxyacetic acid herbicides (2,4-D and 2,4,5-T) are routinely determined by high performance liquid chromatography (HPLC) in combination with GC detection; the analyte is usually derivatized before the GC step. The first of several IAs for the phenoxy herbicides was described by Rinder and Fleeker whose RIA used an antiserum that was raised against the 5-NH_2 derivative of 2,4-D (Figure 9)(Table 1).[170] The assay was more sensitive to 2,4,5-T than to 2,4-D (Table 4) most likely because the ABs recognized the NH_2 group as a chlorine atom. A phenolic derivative of 2,4-D was labeled with ^{125}I by means of a chloramine-T procedure. The ABs bound the radioligand despite the tracer's proximity to the parent molecule; however, the RIA's performance varied with each batch of radioligand.[171] In an effort to improve the specificity of the IAs for 2,4-D, Fleeker[171] raised sera to immunogens that were prepared by conjugation of the carrier protein to (1) the acetic acid moiety and (2) the NH_2 group of 2-chloro-4-aminophenoxyacetic acid (Table 1). The former serum was insensitive to 2,4,5-T, but the latter had comparable avidity for 2,4,5-T and 2,4-D, which indicates that the immune response lacked selectivity in the region of the hapten's conjugation site. Used together, the sera were able to reduce the incidence of false-positive results.

Knopp et al.[172] generated a low titer (1:50) serum to a 2,4-D hapten that was conjugated through the acetic acid group to the carrier protein (Table 1). 2,4-D was unable to compete for antibody binding sites with an ^{125}I-tyramine derivative of 2,4-D,[172,173] probably because the radioligand and immunogen were too similar. A sensitive assay was developed, however, using tritiated 2,4-D as radioligand (Table 4). The highly selective serum discriminated against 2,4,5-T (CR% = 9).[172] The authors suggested that the serum's titer and avidity could probably be improved through the use of a different spacer group in the immunogen. The acetic acid group also was used by Hall et al.[174] as a conjugation site in the production of an anti-2,4-D serum that showed little cross-reactivity toward 2,4,5-T (11%) and monochlorophenoxyacetic acid (16%). A novel radioligand was prepared for use in this assay by coupling tritiated glycine to 2,4-D via a mixed anhydride reaction. The serum also was used in an EIA for 2,4-D.

2. Triazines

Dunbar[175] and Huber[176] concurrently developed a pair of IAs for atrazine (Figure 9), a popular herbicide that is used for the pre- and post-emer-

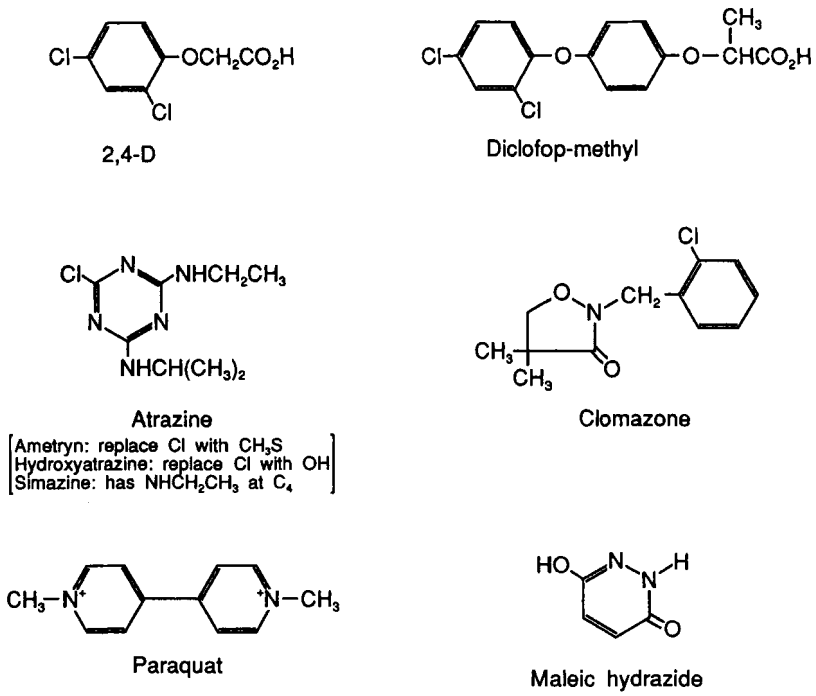


FIGURE 9. Molecular structures of phenoxy acid herbicides, triazines, paraquat, and other herbicides.

gence control of annual weeds in a variety of crops including corn (maize). Dunbar's serum, which was initially described in a patent application,[175] was raised against a hapten that was conjugated through the 4-position on the aromatic ring to a carrier protein (Table 1). A tyrosine methyl ester derivative of the hapten, suitable for labeling with ^{125}I, was synthesized using the mixed anhydride method. For reasons of convenience, an indirect EIA format was pursued.[177] The ABs cross-reacted significantly with propazine (87%) and azido atrazine (58%); simazine (10%) and ametryn (7%) cross-reacted weakly. Deethylatrazine (6%) was the only one of five atrazine metabolites, including hydroxyatrazine, to cross-react in the assay. The assay was judged suitable for the rapid screening of samples for atrazine and propazine.

Huber's version of the atrazine EIA used a serum that was raised against the sulfoxide derivative of ametryn: ametryn is an analog of atrazine (Figure 7; Table 1).[176,178] Purification of the ABs by immunoaffinity chromatography (IAC) improved the assay's detection limit 100-fold (Table 4). When the ABs were immobilized on polystyrene spheres, it was possible to analyze larger sample portions (20 ml) with a corresponding improvement in the assay's detection limit (Table 4). Ametryn (Figure 9) was the most reactive (106%) of several triazine herbicides that were detectable by the assay. An attempt by Sharp et al.[179] to improve the selectivity of anti-atrazine sera by separation of the hapten from its carrier protein with a linkage group was not fully successful; apparently, the serum cross-reacted with triazine herbicides that had similar N-alkyl substituents. Researchers at Shell Development Co. are reported to have developed a highly specific anti-cyanazine serum (Table 1), which was used in a sensitive assay.[137,180] The assay did not cross-react with other commercial triazine herbicides, but could detect metabolites of cyanazine.[137]

Huber and Hock employed their previously proven techniques to develop an EIA for terbutryn (Table 1).[181] Terbutryn is a post-emergence triazine herbicide that is used with several winter cereals; it also is effective in the control of aquatic weeds and algae. Several triazine herbicides and terbutryn metabolites such as hydroxyterbutryn cross-reacted in the assay. The assay's DL was improved 250-fold when the polystyrene sphere format, which is well suited to water analysis, was used (Table 4). The improvement was realized without any pretreatment of the sample.[182]

More recently, Wittmann and Hock used an immunogen that contained 35 atrazine substituents per molecule to generate a serum that was used in a very sensitive assay (Table 1).[183] Immunogens with a range of substitution ratios were produced by varying the type of carbodiimide coupling agent, the hapten/BSA ratio, and the carbodiimide ratio. An immunogen with four atrazine residues per molecule of BSA was used for an initial immunization period of 5 months, at which time the 35-substituent immunogen was employed as a booster treatment. The assay was selective; it cross-reacted with only atrazine and propazine when serum C2 was used. The choice of tracer system was found to influence assay specificity; cross-reactivity with simazine (Figure 9) was particularly affected (max. CR% = 20). Hock's group subsequently published an evaluation of three versions of the atrazine EIA that were based upon different combinations of two sera and two tracers.[184] The sera for this study were raised against two immunogens. The most sensitive assay cross-reacted with propazine (195%) and simazine (20%). Wittmann and Hock have also developed a sensitive EIA for deethylatrazine and deisopropylatrazine, which are key atrazine metabolites.[185] The serum was raised against the 2-aminohexanecarboxyllic acid deethylatrazine immunogen (Table 1), which was prepared using a CMC coupling reaction. The enzyme-labeled hapten was prepared using a CDI/NHS active ester procedure (Table 1). Microtiter plates that were coated with affinity-purified ABs tended to lose activity after 2 weeks storage at 4°C. Serum-coated plates, however, were stable for at least 2 months. Immunogens with high and low hapten substitution ratios were again alternated in the immunization protocol, which again produced a sensitive serum of high titer. The assay could efficiently detect both target compounds. The problem of limited serum supply was tackled by Wust and Hock, who raised large quantities of anti-atrazine sera in sheep (Table 1).[28] The sheep sera were most sensitive for propazine, but did not cross-react with other triazines.

A direct competitive EIA has been used to monitor atrazine and deethylatrazine levels in soils from the Munich area of Germany.[186] The sensitive assays (Table 4) showed little cross-reactivity to other triazine residues; the atrazine assay only cross-reacted with propazine and the assay for deethylated atrazine only cross-reacted with deisopropylated atrazine.

Two versions of an enhanced luminescent IA for the detection of atrazine and simazine have been described.[187,188] In one version, peroxidase-labeled hapten was allowed to compete with the analyte for binding sites on AB-coated polystyrene tubes. The tubes were then washed and the amount of peroxidase bound to the ABs was measured using an isoluminol reagent. A microtiter version of the assay could be developed if a suitable plate reader were available. The reagent concentrations were increased so as to shorten the incubation time and render the assay suitable for field use. An outline report also has been made of an FIA for the quantification of triazine herbicides,[189] for which the tracer was prepared by labeling hapten molecules with fluorophores such as dansyl chloride and fluorescein. Ulrich and Niessner used a fluorogenic substrate for alkaline phosphatase in a direct FIA for the determination of terbuthylazine, which is being used as a replacement for atrazine, now banned in Germany.[191] The MAB-based assay required a 20-h incubation of the substrate and AB-bound tracer. In a later version of the assay, an improved enzyme-tracer conjugate enabled the incubation period to be shortened to 20 min.[192] An NHS-activated ester pathway best combined ease of synthesis and tracer quality.[193] The assay had a slightly lower DL than the corresponding EIA (Table 4).

A simple, rapid, and sensitive tube-based direct EIA for atrazine and other triazines that is suitable for field use is available from ImmunoSystems Inc. The serum used in this assay was raised against an immunogen that had a high substitution ratio of 30:1 and was prepared by derivatization of atrazine at the 2-chloro position (Table 1).[194] The antiserum cross-reacts with several triazine herbicides, which is advantageous when samples are to be screened for the triazenes as a herbicide class. A microtiter strip version of the assay offers superior sensitivity (Table 4) and is more convenient for laboratory use. The assay has been found to be insensitive to variations in pH, temperature, and concentrations of calcium, sodium, and nitrate.[195]

Schlaeppi et al. developed some highly specific anti-triazine MABs against haptens that were prepared by coupling valeric acid derivatives of atrazine and hydroxyatrazine (Figure 9) to carrier protein (Table 1).[196] Two groups of anti-hydroxyatrazine MABs were generated; the first group only cross-reacted with hydroxypropazine, whereas the second group cross-reacted with several other hydroxy-S-

triazines. The anti-atrazine MABs all had comparable selectivity; they recognized propazine (CR% = 90) and had reduced cross-reactivity to other triazine herbicides and their hydroxylated metabolites. Giersch and Hock conjugated ametryn sulfoxide and dichloroatrazine to BSA in order to raise anti-atrazine MABs (Table 1).[197] Four MABs were selected for detailed characterization. The MABs raised against ametryn sulfoxide had strongest affinity for terbutryn and prometryn, whereas the MABs raised against dichloroatrazine were most sensitive to aziprotryn. The selected clones had fairly broad specificity patterns.

Goodrow and co-workers tackled the issue of triazine assay selectivity by preparing a library of atrazine and simazine haptens (Table 1) that could be used to tailor assay specificity and sensitivity to suit analytical needs.[198,199] The haptens were used in the production of both PABs and MABs. One group of haptens, prepared by replacing an N-alkyl group with linear amino acids (C = 1-5), induced ABs that were highly specific for atrazine and simazine. A second group, prepared by substituting the 2-Cl of atrazine or simazine with 3-mercaptanpropanoic acid, induced PABs that cross-reacted with the S-methyl triazines. The degree of analyte binding was found to depend on the hapten's structure and on the position and length of the immunogen's spacer arm. The specificity of sera from replicate rabbits varied widely. This library of antibodies and coating haptens has been used in several sensitive and versatile triazine IAs. Both heterologous and homologous assay formats have been evaluated. The heterologous assays were more sensitive to the target analyte. The coating antigen's conjugation site had the greatest influence on assay sensitivity. The sensitive heterologous assay systems, however, were more susceptible to interference by residual solvents and matrix components — which implies it may sometimes be necessary to strike a balance between assay ruggedness and sensitivity. The MABs were used as the basis of a sensitive assay for the detection of atrazine and cross-reacting S-triazine herbicides.[200] A variety of assay formats thatincluded the use of an immobilized double antibody (IDAB) assay were evaluated. The IDAB format is described in the section of the report that deals with IAs for fungicides. A suite of four different haptens were evaluated in the assay in combination with two enzyme tracers. The IDAB format was found to have lower I_{50} values than the corresponding direct EIAs. The use of C6 spacer arms between the enzyme and hapten yielded better calibration curves than C3 arms in the case of horseradish peroxidase (HRP); the reverse was true for alkaline phosphatase. Overall, the C6-HRP combination seemed to provide the best assay. This work is significant because it demonstrates that even the performance of highly selective MABs can be affected by the hapten-label structure. The use of protein A as the primary coating layer also was evaluated because of its ability to orient the capture ABs in a configuration that is favorable for binding to ligands. The protein A-based assay was slightly more sensitive than the IDAB version. The assay cross-reacted with simazine, ametryne, propazine, terbutylazine, and atrazine mercapturate.

3. Paraquat

The frequent implication of paraquat (Figure 7) in poisoning incidents created a need for a rapid and cost-effective method for the determination of this broad spectrum herbicide in human serum. Levitt reported the production of anti-paraquat ABs, which were used in a sensitive and selective RIA (Table 4).[201] An optimized 30-min assay version had a reported sensitivity of 12 pg/100 µl.[202] Fatori and Hunter developed two versions of an RIA for the detection of paraquat in serum and aqueous samples.[203] One version used tritiated paraquat as radioligand and was most suited to rapid clinical applications. A more sensitive version, based on an iodinated tracer, was slower (2 h) but could detect lower concentrations (Table 4). Niewola and coworkers at Imperial Chemical Industries (ICI) developed and systematically optimized an indirect EIA for paraquat (Table 1).[204] Diethylparaquat (40%) and monoquat cross-reacted in the assay.[205] Unfortunately, the ABs in later bleeds from the same animal bound the enzyme-hapten conjugate so strongly that it could not be displaced by free hapten. This setback prompted a decision to develop anti-paraquat MABs so as to ensure a consistent supply of ABs.[205] The MAB-based assay had a steeper calibration curve, although its specificity was similar to that of the original assay; once again, diethylparaquat cross-reacted significantly (>100%).[205,206] The anti-paraquat MABs were later purified by affinity chromatography in order to remove unwanted monovalent ABs.[207] A version of

the assay suitable for the analysis of paraquat in soil has been described.[206] Nagao et al. used the same hapten as the ICI team to develop anti-paraquat MABs (Table 1) that had much reduced cross-reactivity to diethylparaquat (8%) in a heterologous EIA; unfortunately, this assay was less sensitive than the ICI group's (Table 4) homologous assay.[208] Johnston et al. used *in vitro* and *in vivo* immunization techniques to prepare IgM and IgG MABs to paraquat;[209] the selective ABs weakly cross-reacted with monoquat.

Hammock's team at the University of California (UCLA) also undertook extensive research that led to a PAB-based EIA for paraquat.[210-212] They evaluated four carrier proteins in their assay; conalbumin (CONA) and keyhole lymphocyte albumin (KLH) were the best immunization and coating AGs, respectively. A valeric acid linkage system worked best with both AGs (Table 1). Particular care was paid to the structure and concentration of the coating AG; assay sensitivity is usually enhanced when the ABs do not preferentially bind the tracer or immobilized ligand. The serum and sample were preincubated for 24 h. The ABs cross-reacted strongly with the methylpropyl analog of paraquat, but showed low recognition for diethylparaquat and other potential interferences.

Coxon and co-workers used high-affinity ABs (1.6×10^8 to 1×10^9 mol/l) that were raised in sheep to develop a sensitive competitive binding FIA for paraquat (Table 4).[213] Emphasis was placed on assay speed because of the importance of timely data in clinical cases involving the ingestion of paraquat. The first and second ABs were pre-equilibrated so as to shorten the assay's duration to less than 30 min. The serum did not cross-react with diquat and only weakly recognized monoquat (CR% = 4.3).

4. Other Herbicides

Both FIA and EIA assays have been developed for the detection of the weed grass control agent, diclofop-methyl (Figure 9).[214] The hapten was labeled with fluorescein and enzyme labels by means of mixed anhydride reactions. The AB's inability (Table 1) to differentiate the stereoisomers of diclofop-methyl should not be a problem in environmental tasks. Diclofop acid and a 2-methoxy-1-methyl-2-oxoethyl ester of diclofop acid cross-reacted significantly in the assay. The related herbicides 2,4-D and dichlorprop did not cross-react, probably because they lack the 4-(2,4-dichlorophenoxy)phenoxy moiety.

Clomazone (Figure 9), the active ingredient of the preemergence/plant herbicide, Command, is used mainly to control grass and dicot weeds in soybean crops. Clomazone is typically determined by GC-based methods after extensive sample work-up.[215] An EIA for the detection of clomazone in soil extracts (Table 1) did not cross-react significantly with metolachlor, metribuzin, or trifluralin, three herbicides that are often used in combination with clomazone.[215] The FMC Corp. (New Jersey) also has developed an EIA for the measurement of Clomazone in soil (Table 1).[216] Their ABs were most specific for the isoxazolidinone ring structure, and did not cross-react with a broad range of herbicides that might be found in association with Clomazone.

Monsanto Agricultural Company has used an EIA to screen water samples for the presence of Alachlor (Figure 10). Alachlor is the active ingredient of the widely used Lasso formulation and is also a component of the herbicide mixtures Bronco and Alazin.[217] Thiolating reagents (Table 1) were used to conjugate Alachlor to BSA (immunogen) and sheep IgG (coating AG) by heterologous linkages. The serum and sample were pre-incubated for 1 h. Systematic optimization of the coating AG and serum levels resulted in a sensitive (Table 4) assay that was acceptably precise over the assay's working range (CV = 4.2% at 0.2 ppb and 18.6% at 8.0 ppb). The selective ABs only weakly recognized some other chloroacetanilide herbicides, probably because the immunogen had been prepared by linking the carrier protein to the hapten's chloroacetamide group; that group is common to the chloroacetanilide herbicides. Some thioether analogs of Alachlor that are formed as animal metabolites did cross-react in the assay, but are unlikely to be found in the aquatic environment and should not detract from the assay's usefulness as a screening tool. The Monsanto researchers also have reported the development of EIAs for three further chloroacetanilide herbicides: metolachlor, amidochlor, and butachlor.[218] As with the alochlor assay, the AGs were prepared by coupling the

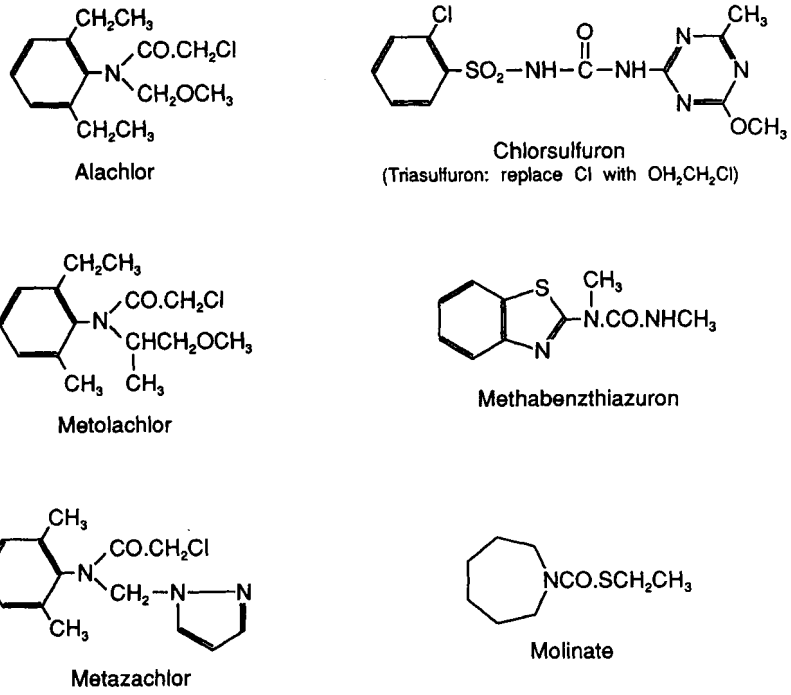


FIGURE 10. Molecular structures of various herbicides.

analytes to carrier protein via thioether linkages. The EIAs were highly specific for the target analytes and did not cross-react appreciably with other structurally related chloroacetanilide herbicides. This high specificity is thought to arise from the preservation of the structurally characteristic N-alkyl groups in the immunogens. The calibration curves for the amidochlor EIA (Figure 11) illustrate how the choice of coating AG can affect an assay's performance. The most sensitive assay system was based on a butachlor-HSA coating AG. When that system is used, the anti-amidochlor ABs, which have low avidity for butachlor, are easily displaced from the solid phase by amidochlor. This strategy resulted in a tenfold improvement in sensitivity.

Ohmicron have adapted their magnetic particle-based technology for the detection of alachlor in water.[219] The technology was developed earlier for use in an EIA for aldicarb.[220] The format is claimed to reduce or eliminate several problems that are associated with conventional EIA formats, such as well-to-well variability and the loss of ABs through leaching. The assay is a direct competitive EIA in which peroxidase-labeled hapten is used to measure analyte-free binding sites. A specially designed magnetic rack is used to retain the magnetic particles during reagent changes. A variety of inorganic parameters did not adversely affect the assay. The robust assay also was unaffected by variations in pH

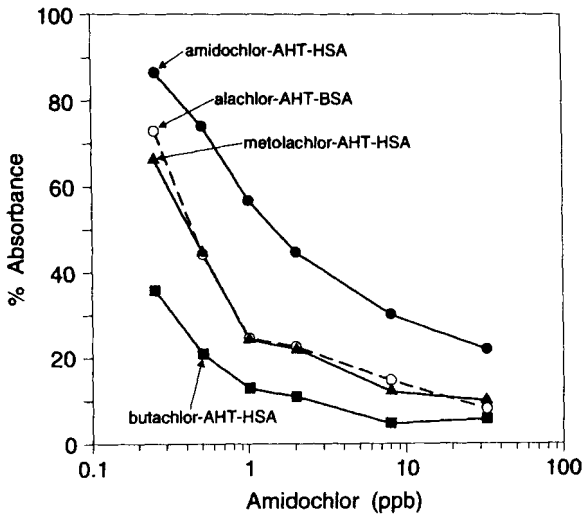


FIGURE 11. Calibration curves for amidochlor EIA illustrate how different coating antigens can affect assay performance. (Reprinted with permission from Feng et al. *J. Agric. Food Chem.* **1992,** *40,* 211–214; American Chemical Society.)

over the range 2 to 12. The antiserum weakly recognized butachlor and metolachlor (CR% of 1 and 1.5, respectively). Researchers at Ciba-Geigy in Switzerland have developed an MAB-based EIA for the related herbicide Metolachlor (Figure 10), which is the active ingredient of the selective herbicide Dual.[221] A distal-carboxylic derivative of metolachlor that was intended to optimize the assay's sensitivity was used to prepare the immunogen (Table 1). Direct and indirect assay versions were evaluated. The assay was highly selective; no significant cross-reactivity was observed with alachlor, furaloxyl, metalaxyl, and several metabolites of metolachlor. The indirect EIA was the slightly more sensitive of the two assay versions.

In order to develop an EIA for Metazachlor (Figure 10), a preemergence herbicide that is used with rape, potatoes, and other crops, the target compound was directly coupled to sulfhydryl groups that were introduced into BSA by cleavage of disulfide bonds with DTT (dithiothreitol) (Table 1).[222] A direct assay format was used in which the PABs were immobilized in microtiter wells. The ABs were highly selective for Metazachlor; several Metazachlor derivatives and chloroacetamide herbicides cross-reacted less than 1%.

Residues of *maleic hydrazide* (Figure 9), a synthetic plant growth regulator, are of concern in beets, potatoes, onions, and tobacco. Harrison et al. described two hybridoma cell lines that produced MABs with high specificity for maleic hydrazide.[223] The affinity-purified MABs were used in an indirect EIA. The MABs cross-reacted significantly with some acetic acid derivatives of maleic hydrazide but did not recognize a variety of purine and pyrimidine compounds. A heterologous assay system was more sensitive, but also was less tolerant of sample variability (pH, ionic strength, and residual solvent) than a homologous system; such interferences should be readily controllable in water samples. Later evaluation of the assays using potato extracts showed that the heterologous format did not offer a significant advantage — mainly because it yielded more variable data.[224]

A du Pont research team developed a PAB-based EIA for the detection of *chlorsulfuron* (Figure 10), the active component of Glean Herbicide.[225] The samples and serum were preincubated for 1 h. Chlorsulfuron's main subunits did not cross-react in the assay. Although two herbicides that closely resembled chlorsulfuron's bridge and heterocyclic ring structure did cross-react in the assay, two other related herbicides with different bridge and heterocyclic ring structures had greatly reduced cross-reactivity.

An extremely sensitive MAB-based assay for triasulfuron (Figure 10) was developed by Schlaeppi et al. at Ciba-Geigy Ltd.[226] Rearrangement problems with the spacer group on a hapten that closely resembled the entire target molecule (Table 1) resulted in specific but insensitive MABs. MABs raised against a hapten that was based on the chloroethoxy phenylsulfonamide carboxy portion of triasulfuron were sensitive (Table 4) and specific. The ABs and analyte were preincubated for 1 h. The MABs selected for use in the screening assay cross-reacted with only the phenylhydroxylated degradation products of triasulfuron.

A study was initiated by Health and Welfare Canada to produce antibodies to the phenylurea (carbamide) herbicides.[227] Several immunogens (Table 1) were used to induce serum production in rabbits. Owing to the overly strong binding of ABs to the coating AGs, it was necessary to use coating AGs that were heterologous with respect to the immunogen. The various serum coating AG combinations were systematically screened for cross-reactivity, and a combination was selected that yielded workable assays for monolinuron, diuron, and linuron (Table 4). Several related urea compounds such as neburon and metabromuron cross-reacted in the assay.

A sensitive IA has been described for the urea herbicide methabenzthiazuron (MB) (Figure 10).[228] The PAB-based assay (Table 1) uses a direct competitive format, and it has impressive assay performance characteristics (Table 4). The assay also detected OH-MB (CR% = 50) (OH-MB is a decomposition product of MB) and benzthiazuron (CR% = 2). Hock's group at the Technical University of Munich, who originated the foregoing assay are now working on the production of MABs to methabenzthiazuron.

Concern about residues of the selective herbicide molinate (Figure 10) in drainage canals and receiving rivers near rice paddies prompted the development of a PAB-based EIA.[229] A variety of haptens were coupled to both the immunization and

coating proteins. The conjugation site and the type and length of the linkage arm were varied (Table 1); several rabbits were inoculated with each immunogen. This multipronged approach increases the likelihood that the optimized assay will be sensitive and can enable the analyst to fine tune the assay's specificity. Sera were raised against carboxyethyl, carboxypentyl, and aminobenzyl derivatives of molinate (Table 1). The samples and standards were preincubated with serum for 16 h. Sera that had greater avidity for the coating AG than the free analyte were discarded. An S-2(*p*-aminophenyl)ethyl derivative of molinate coupled to CONA was selected as the coating AG for the optimized assay; the selected serum was raised against S-2-carboxyethyl molinate. The assay was highly specific for molinate, although some cross-reactivity (15%) with molinate sulfone was observed.

Hall and co-workers developed an RIA for picloram (Figure 12),[174] an auxin-type herbicide that should be monitored in surface and receiving waters adjacent to application areas. Picloram coupled to [^{3}H]glycine was used as radioligand. None of the related herbicides that were tested could inhibit tracer binding by 50%. These researchers later developed anti-picloram MABs (Table 1), which were used in an effective EIA.[230] Both MAB- and PAB-based versions of the EIA were more sensitive than the previously developed RIA. Neither the PABs nor the MABs cross-reacted appreciably with 2,4-D or any of the pyridine herbicides that were tested. The induction of low-affinity PABs and high-affinity MABs against the same immunogen implies that immunogen design and preparation are less critical for the production of MABs.

Amitrole (MW, 84.1; Figure 12) is a nonselective herbicide that is used in fruit orchards and in the preparation of soil for some crops, including kale and maize. Jung et al.[231] reported efforts to develop an EIA for the detection of this water-soluble analyte. A library (Table 1) of antigens were used to immunize both rabbits and mice. The sera, which were expected to have low avidity because of the hapten's small size, were screened using an indirect solid-phase EIA. A promising serum was selected for use in an optimized heterologous assay. The pH of the incubation mixture affected the binding properties of the highly specific ABs. The low sensitivity of the assay (Table 4) suggests that amitrole was poorly recognized by the host's immune system. This study

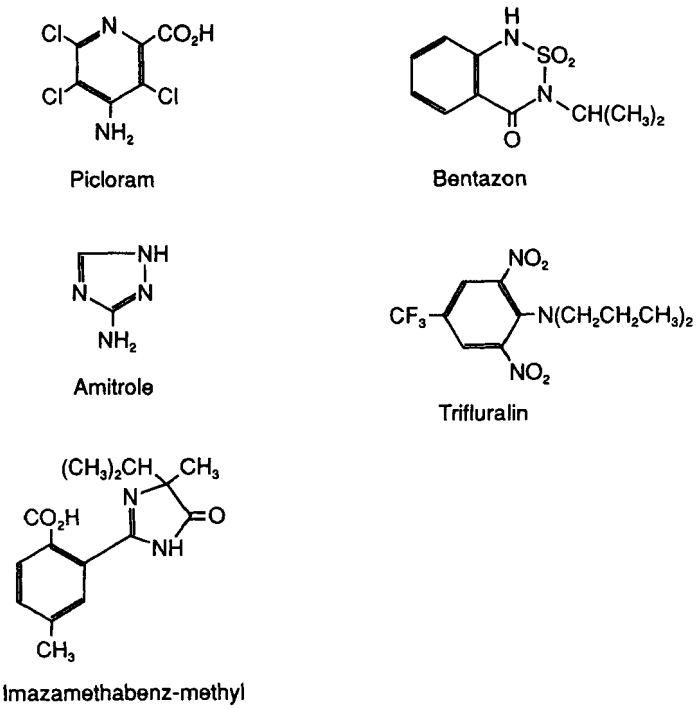


FIGURE 12. Molecular structures of various herbicides.

illustrated some of the problems to be expected in the development of IAs for very small analytes.

Because the post-emergent herbicide imazamethabenz-methyl (Figure 12) is hydrolyzed by plants to the free acid, Newsome and Collins opted to develop ABs to imazamethabenz for use in an indirect EIA.[232] The assay was selective for the target analyte and its methyl ester (CR% = 627). Wong and Ahmed from the American Cyanamid Company have reported the development of a sensitive direct competitive EIA for the determination of imazaquin, which is the active ingredient of the herbicide Scepter.[233] A heterologous bridge was used to link the HRP tracer to the hapten. A variant of the IDAB format, in which protein A was used to bond the primary ABs to the solid phase, was used. The assay can detect the related imidazolinone herbicides such as imazapyr and imazaquin, which yielded calibration curves that were similar in shape to the imazaquin curve but shifted to the right. A variety of metabolites of the target herbicide and a broad range of agrochemicals thought likely to be present in real world samples did not interfere with the assay. The preparation of a suitable immunogen for use in the generation of sera against the sulfonamide herbicide bentazon (Figure 12) presents a special problem because bentazon contains an ionizable NH group.[234] Immunogens were prepared by coupling the hapten to its carrier protein through the NH group and the aromatic ring. Only the immunogens that were coupled through the NH group using a spacer molecule yielded ABs that could detect both bentazon and its N-alkylated derivatives. The immunogens that were coupled through the aromatic ring yielded ABs that recognized N-alkylated derivatives of bentazon but not the free compound. A wide range of herbicides failed to cross-react in the assay.

Riggle published an account of an insensitive and poorly selective EIA for the preemergence herbicide trifluralin (Figure 12).[235] The authors postulated that the use of a larger bridging group in the immunogen might improve the serum's specificity. The first IA for a phenylpyridazinone-based herbicide was reported by Riggle and Dunbar.[236] Two anti-norflurazon sera cross-reacted with the closely related compounds desmethyl norflurazon and metflurazon. Several non-phenylpyridazinone-based herbicides that were tested did not cross-react in the assay.

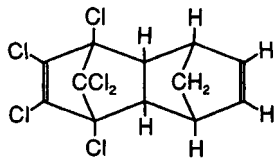
D. Insecticides

1. Chlorinated Hydrocarbons

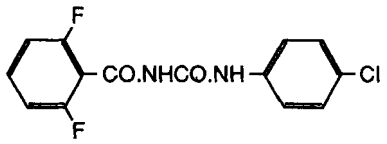
The first IA for an insecticide was designed by Langone and Van Vunakis to detect aldrin and its metabolite dieldrin (Figure 13).[122] Aldrin is an organochlorine compound that has broad-spectrum activity. The immunogen was prepared from a carboxylated derivative of aldrin (Table 1). The same derivative was coupled to tyramine and labeled with ^{125}I (Table 5) to yield a radioligand of low specific activity (3.3 Ci/mmol). The analyte was solubilized in horse serum (10%). Compounds that resembled the immunogen's distal region tended to cross-react in the assay. Heptachlor and chlordane competed 13 and 26 times, respectively, less effectively than dieldrin. Endrin cross-reacted significantly, but because of its short half-life was considered unlikely to interfere in the analysis of physiological fluids. DDT and CL_{10}-PCB also cross-reacted (20%).

A tube-based EIA suitable for field applications has been developed for the detection of chlordane — another of the cyclodiene insecticides (Figure 13).[237] Inasmuch as the assay detects other cyclodiene insecticides such as dieldrin, aldrin, endrin, endrin ketone, chlordane, and endosulfan, it is best used as a general screen for the cyclodienes. This assay should be readily adaptable for use with water samples.

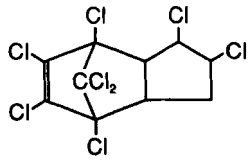
Dreher and Podratzki generated an anti-endosulfan (Figure 13) serum by using a succinate bridging group to separate the hapten from its carrier protein (Table 1).[238] The preparation of enzyme-labeled hapten was complicated by endosulfan's hydrophobicity, which caused the peroxidase label to precipitate. The problem was solved by synthesizing an amine derivative of endosulfandiol, which had reduced hydrophobicity. That derivative was then coupled to the peroxidase enzyme; however, the periodate coupling reaction was only able to couple 20 to 30% of the enzyme molecules to the hapten.



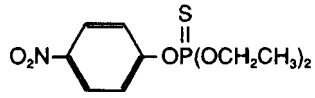
Aldrin



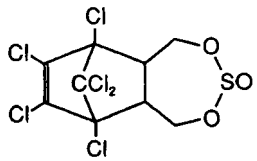
Diflubenzuron



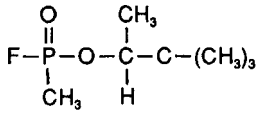
Chlordane



Parathion
(Paraoxon: replace S with O)



Endosulfan



Soman
(MATP: replace F with NH_2ArO)

FIGURE 13. Molecular structures of various insecticides.

The EIA was more sensitive for endrin (CR% = 180) than for endosulfan; aldrin had much lower cross-reactivity (16%).[239] Several common degradation products of endosulfan were detectable in the same concentration range as the target molecule.

2. Pyrethrins

The potency, low mammalian toxicity, and short environmental half-life of the pyrethrins are environmentally friendly traits. Nonetheless, the pyrethrins are difficult to analyze because of their instability in heat and light.[240] Such considerations prompted the production of ABs for use in an RIA for S-bioallethrin.[241,242] The radioligand was prepared by coupling [^{3}H]tyramine to the hemisuccinate derivative of an allethrin alcohol. The ABs were able to distinguish the optical and geometric isomers of allethrin. Similar selectivity was observed when the serum was subsequently used in an EIA.[243]

Investigators at Shell Research Ltd. have developed two variants of a pyrethrin EIA directed against 3-phenoxybenzoic acid (PBA) and dichlorovinyl cyclopropane carboxylic acid (CYP), which are plant metabolites of cypermethrin.[137,244,245] The assay based on anti-PBA antibodies could detect a broad range of pyrethroids and metabolites that contained the PBA moiety. The second serum, which was prepared against CYP, was used to detect cypermethrin and permethrin. Organic solvents were used at levels of up to 30% to help solubilize the analyte in buffer. Some solvents were observed to improve the calibration curve's slope. A MAB-based version of the PBA assay also was developed.[137]

Stanker and colleagues at Lawrence Livermore National Laboratory developed three anti-pyrethroid MABs against acidified phenothrin (Table 1).[240] The hapten's conjugation site was located as far as possible from the phenoxyphenyl group in an effort to maximize the MAB's specificity for that group; the phenoxyphenyl group is common to several synthetic pyrethroids. 3-PBA-BSA was used as the coating antigen. Initial evaluation studies led to the selection of clone PY-1 for further study because of the specificity and sensitivity of its MABs, which could distinguish between several closely related pyrethroids.

3. Benzoylphenylureas

The benzoylphenylurea insecticides are nonvolatile and difficult to analyze unless they are derivatized. Wie and collaborators were the first to research the development of IAs for diflubenzuron (Figure 13) and BAY SIR 8514.[246] Several haptens were designed with the goal of optimizing the immune system's recognition of the analyte's benzamide subunit (Table 1).[246] A carboxypropyl derivative of diflubenzuron was used to induce several useful sera, one of which was used to develop three EIAs for the detection of diflubenzuron, BAY SIR 8514, and some of their analogs.[247] The analyte was preincubated with the serum for 16 h. Of two rabbits that were immunized with carboxypropyldiflubenzuron, one produced ABs that bound diflubenzuron, BAY SIR, and like compounds; whereas, serum from the other rabbit was selective for the diflubenzuron group. This observation reinforces the wisdom of raising sera in multiple rabbits. Diflubenzuron phenylacetate-based AGs were superior as coating AGs to the carboxypropyldiflubenzuron AGs because of overly strong binding of the latter by the primary ABs. A simplified version of the assay that used labeled ABs had similar sensitivity to a conventional indirect EIA (Table 5).

In a follow-up study, the effect of bridge recognition on assay sensitivity was explored.[248] Three approaches were used: homologous sites on the hapten were used to link the immunogen and coating AG to their carrier proteins via heterologous bridges, and heterologous conjugation sites were evaluated with both homologous and heterologous bridges. The heterologous assay systems were the more sensitive (Table 5) and the choice of coating AG was shown to affect the assay's sensitivity and working range. The structure of the immunogen and coating AGs also influenced assay specificity. The study showed that a library of ABs and coating AGs can be used to tailor assay sensitivity and selectivity so that single compounds or a group of related compounds are detected. This strategy has been repeatedly used by Hammock's group to successfully develop IAs for a variety of residues.

4. Organophosphates

Parathion (Figure 13) is widely used for the control of soil-dwelling insects. Most matrices require a lengthy clean-up for the determination of parathion residues by gas liquid chromatography (GLC). Ercegovich et al. developed RIAs for parathion that used both [^{14}C]ethylparathion (40 mCi/mmol) and ring-labeled [^{3}H]parathion (300 mCi/mmol) as radioligands (Table 1).[249] The PABs were highly selective for parathion; only reduced parathion cross-reacted appreciably. A radioligand with higher specific activity would probably help improve the assay's detection limit (4 ng). Surprisingly, attempts to induce a more avid and selective antiserum by separation of the hapten from its carrier protein were unsuccessful.[250] Parathion's nitro group was preserved in three of the tested immunogens with the intent of improving the assay's selectivity. The resultant ABs were unable to bind free parathion, although they could bind various hapten-bridging group conjugants. The original diazo-linked immunogen may well be optimal for the induction of anti-parathion ABs.[249] It is also possible that parathion's small size presents special problems.

Lober et al. reported the production of antisera to paraoxon (Figure 13), which is the primary oxidation metabolite of parathion.[251] The antisera cross-reacted with parathion (CR% = 8.8 to 13.8) and methylparathion (CR% = <1). Hunter et al. developed a sensitive EIA for paraoxon.[252,253] The assay, which was intended as a diagnostic aid, used IAC-purified ABs that were preincubated with the analyte for 1 h. The assay's calibration curve was linear to 10^{-10} *M* (Table 5); parathion was only weakly (CR% = <1) recognized,[253] as were *p*-nitrophenol (CR% = 0.04) and diethyl phosphate (CR% = 0.7), which are hydrolysis products of paraoxon.[254] Brimfield et al.[254] were motivated by the vagaries of animal lifespan and immune response maturation to undertake the production of a stable supply of anti-paraoxon MABs. The immunogen was again prepared from reduced *p*-NH_2-paraoxon (Table 1). The MABs from two hybridoma lines were purified by affinity chromatography and used in an indirect competitive EIA (Table 5). The assay calibration curves for the two MAB lines (Figure 14) indicate that the MABs were less sensitive than the earlier PABs (Table 5). Significantly, both sets of MABs did not recognize either *p*-nitrophenol or diethyl phosphate, or similar insecticides. The MABs did however cross-react with (*p*-aminophenyl)paraoxon (CR% = 127), parathion (CR% = 3.6), methyl parathion (CR% = 3), and diethyl phenylphosphonate (CR% = 4.4). Anti-paraoxon sera also have been

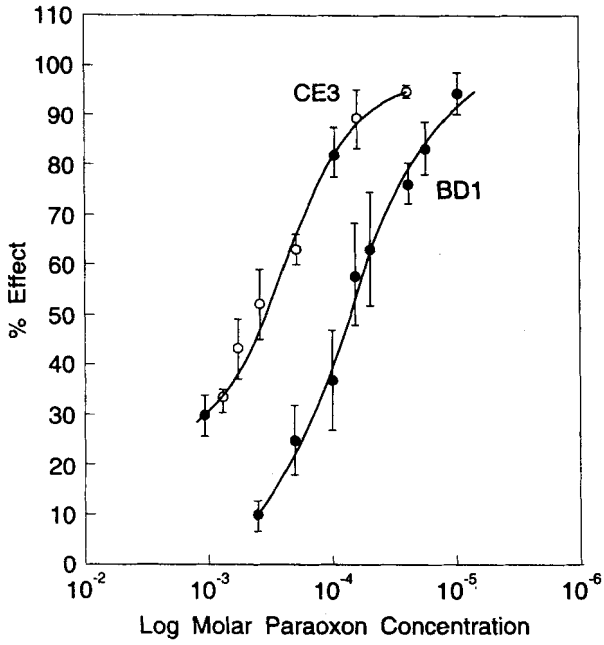


FIGURE 14. Calibration curves for paraoxon: MAB-based assay. (Reprinted with permission from Brumfield et al., *J. Agric. Food Chem.* **1985,** *33,* 1237–1242; American Chemical Society.)

used in a novel IA format that is based on competition between acetylcholinesterase (AChE) and ABs for free paraoxon.[255] Since the toxic effects of organophosphorous insecticides are thought to be caused by the inhibition of AChE activity, it was postulated that the anti-paraoxon ABs could be used to protect AChE *in vivo*. The immunogens for these experiments were prepared by linking paraoxon to carrier protein through the phosphorus moiety, thus preserving the nitrophenol group (Table 1). The sera, which also were used in an RIA, recognized parathion but did not cross-react with *p*-nitrophenol or diethylphosphate. The ABs had higher affinity than AChE for paraoxon and were able to reduce paraoxon-induced inhibition of enzyme activity *in vitro*. The ABs were used to reduce the toxic effects of paraoxon on mice in some *in vivo* tests.

A concerted effort to develop a group-specific IA for the detection of the diethyl ester of phosphates, thiophosphates, dithiophosphates, and phosphonates was undertaken by Sudi and Heeschen.[256] Since the majority of organophosphorous insecticides have either $(EtO)_2P(S)Y$ or $(MeO)_2P(S)Y$ structures, IAs targeted against these groups would be a valuable asset. The ABs for use in such IAs should discriminate against the Y group. The role of the capture AG on overall assay specificity was addressed. Three antiserum/capture AG systems were studied in some detail. DCP(0,0-diethyl-0-[4-carboxyethyl-phenyl] phosphate) linked to polylysine and directly phosphorylated polylysine were selected for use as the capture antigens. The three serum (Table 1)/capture AG combinations chosen yielded sensitive assays that had broad specificities. The specificities of the assay systems was evaluated using 58 organophosphorous compounds. Diethyl esters were about 100 times more reactive than the analogous dimethyl esters. An homologous DCP-based assay system was more selective than the heterologous assays. The specificity patterns of the three systems were partially nonoverlapping and depended on the immunogen and capture AG used. Consequently, the experimental data were reevaluated on the basis of "double test" assay systems. The best combination of tests could detect 83.3% of the phosphorus diethyl ester compounds that were tested at an estimated DL of 54 μg/ml. The authors recommended further research on the use of the "double test" system for the detection of dimethyl and diethyl phosphate derivatives in environmental samples.

Bio-metric Systems Inc. market an EIA that is designed for the easy detection of paraoxon in the field.[257] Enzyme-labeled hapten was prepared by coupling diethyl 4-aminobenzylphosphorate to carrier protein via a succinate spacer group. The reagents are incorporated into a "pinch test" format that is ideal for unskilled personnel. In this format, the ABs are immobilized on a porous disk to which up to 1 ml of sample can be added. Enzyme-labeled hapten is then added and a substrate disk is pinched into contact with the porous disk. The developed color is read after 5 min.

Hunter et al. initiated efforts to develop MABs against the warfare agent Soman (Figure 13).[258] The techniques used in this study (Table 1) could be used in the production of ABs to other low molecular weight organophosphorous pesticides, such as glyphosate. The MABs were selective for Soman and did not react strongly with Sarin, a similar compound. Lenz et al. studied the specificity of anti-Soman MABs and PABs.[259] The PABs weakly recognized Soman but cross-reacted strongly with various Soman analogs. The PAB's weak reaction with Soman was attributed to the apparent dominance of the immunogen's hydrophobic *p*-aminophenyl group. On the other hand, the MABs reacted strongly with Soman and were not inhibited

by Sarin or Soman's hydrolysis products. As was the case with the anti-paraoxon serum of Heldman et al.,[255] the MABs could compete with AChE for its inhibitor — which in this case was Soman. The anti-Soman AB's, however, were only marginally effective in preventing the toxic effects of Soman in mice.[264] Schmidt et al. raised anti-MATP sera in chickens and rabbits;[260] the Soman derivative MATP (methyl phosphonic acid, *p*-aminophenyl-1,2,2-trimethyl-propyldiester) (Figure 11) was chosen as a model organophosphorous compound. The AG was covalently bound to microtiter wells using Schiff's base. The rabbit ABs, which were purified by IAC, yielded the more sensitive assay. This research group also produced anti-MATP MABs, which were used in a direct competitive EIA (Table 5).[261] Most of the selected MABs were highly specific for MATP, and the MAB-based assay was less sensitive, but more reproducible, than the PAB assay. The MABs, which could detect free Soman in the EIA, weakly recognized free Sarin but should be able to recognize some nontoxic Soman analogs.[262] The anti-MATP MABs also have been used in direct competitive and indirect competitive CIAs (Table 5).[263] A camera luminometer was used to measure the final signal in a version of the CIA that is suitable for field use. Buenaffe and Rittenberg prepared anti-Soman MABs so as to closely study the AB-Soman interaction. Two distinct groups of MABs were produced that were highly specific for Soman and did not cross-react with the structurally similar molecule phosphocholine.[265] The main difference between these two compounds is the presence of a positively charged choline group in phosphocholine.

Researchers in Australia have developed a set of ABs for the following organophosphorous insecticides: fenitrothion (FN), chlorpyrifos-methyl (CPMe), and pirimiphos-methyl (PM) (Figure 15).[266] The ABs were used in a pseudo-multianalyte IA in which neighboring microtiter wells were individually coated with each AB. The FN assay cross-reacted weakly with some structurally related insecticides such as parathion and dicapthon. CP-ethyl (CR% = 15), fenchlorphos (CR% = 40), and bromophos (CR% = 60) cross-reacted in the CPM

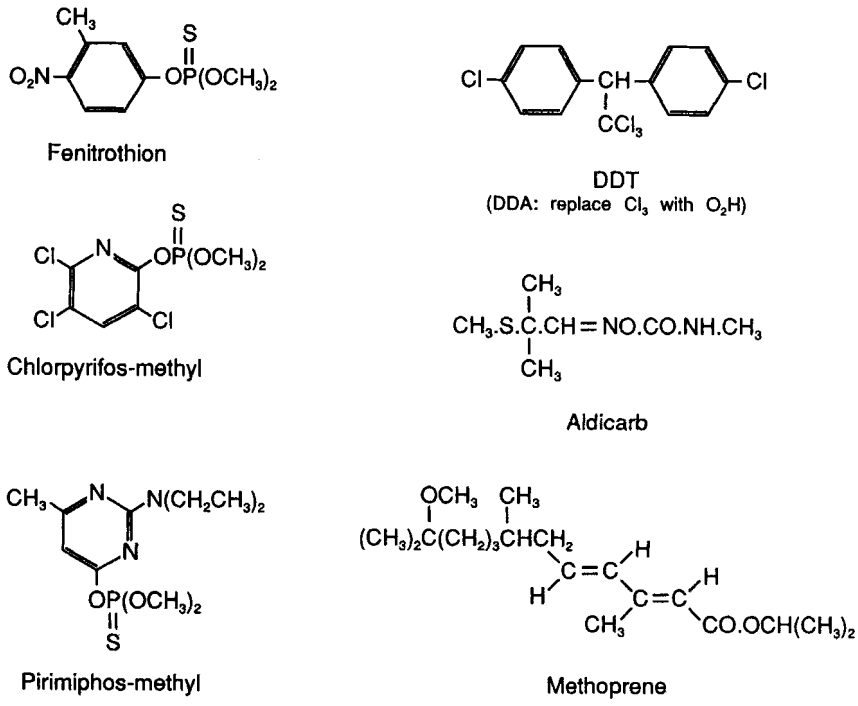


FIGURE 15. Molecular structures of various insecticides.

assay, however that should not be a problem since those compounds are rarely used on grain. The PIRM assay was similarly selective.

5. Other Insecticides

DDA [2,2-bis (*p*-chlorophenyl) acetic acid], the chief urinary metabolite of DDT (Figure 15), is useful as an indicator of DDT exposure in humans. Haas and Guardia raised antibodies to a DDA-protein conjugate (Table 1); the ABs detected free DDA in a hemagluttination test, but did not cross-react with DDT.[121] Centeno et al.[120] used DDA anhydride that was linked directly to a carrier protein to induce anti-DDA antibodies in rabbits (Table 1). That antiserum was able to bind the benyl amine salt of the aminocaproic acid derivative of DDA. Neither of the foregoing anti-DDA sera was used in an IA. Later, however, Banerjee developed an EIA for the detection of DDA in urine extracts (Table 1).[267] The serum and analyte were preincubated for 1 h. DDT, DDE, and DDD cross-reacted less than 5% in the assay.

Aldicarb (Figure 15) is cumbersome to analyze by conventional GC and HPLC methods.[268] A heterologous EIA has been used to detect aldicarb in water, body fluids, and juices. The direct EIA format, which was selected because of its convenience, does not require preincubation of analyte and reagents. The heterologous assay design was effective in the elimination of bridge recognition effects. The highly selective assay had a dose response from 15.6 to 2000 ng aldicarb.[269] Aldicarb sulfoxide and aldicarb sulfone, which are important degradation products of aldicarb, did not cross-react in the assay. An anti-aldicarb serum has been used in a novel assay format in which the serum was covalently immobilized on magnetic particles.[220] The assay had a linear dose response from 1 to 100 ng/ml and was highly specific for aldicarb, aldicarb sulfone (CR% = 5; DL = 0.5 ng/ml), and aldicarb sulfoxide (CR% = 3; DL = 5 ng/ml). The aldicarb EIAs should be particularly useful for the screening of water samples.

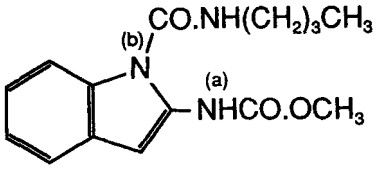
The insect growth regulator methoprene (Figure 15) is used on tobacco crops and to control insect pests of farm animals. A collaboration between ImmunoSystems Inc. and the University of Massachusetts has produced a direct competitive EIA that can be used in the analysis of crop extracts and environmental samples.[270,271] Care was taken to preserve the characteristic ester and methoxy groups of methoprene when preparing the immunogen. The result was a serum that is highly specific for methoprene and cross-reacts significantly only with methoprene acid, which is a microbial degradation product of methoprene in water samples. The assay was sensitive to the inclusion of Tween 20 in the assay reagents. A direct competitive format was more sensitive than its indirect counterpart (Table 5) and also was more resistant to matrix effects when used to assay tobacco extracts.

Bacillus thuringiensis var. kurstaki and *israelensis* make crystalline inclusions that are toxic to insects. Until the development of a suitable EIA, insect bioassay and rocket immunoelectrophoresis were the analytical methods of choice for these biological insecticides.[272] IAs are ideal for the analysis of high molecular weight proteinaceous compounds that are difficult to analyze using conventional techniques. One- and two-step indirect EIAs were developed. The assays had similar sensitivities and wide working ranges of 30 to 3000 ng/ml (two step) and 2 to 200 ng/ml (one step) of endotoxin. The EIA procedure was improved by pretreating the polystyrene cuvettes with gluteraldehyde and using toxin-conjugated enzyme in a direct EIA format.[273] The gluteraldehyde pretreatment is thought to improve the ABs orientation on the solid phase. The modified assay used less serum and had a working range of 10 to 1000 ng/ml. Cheung and Hammock used an indirect EIA with a working range of 15 to 1000 ng/ml to monitor the d-endotoxin of *B. thuringiensis israelensis*.[274] A noncompetitive sandwich EIA has been used to measure *B. thuringiensis* crystal protein during the manufacturing process.[275]

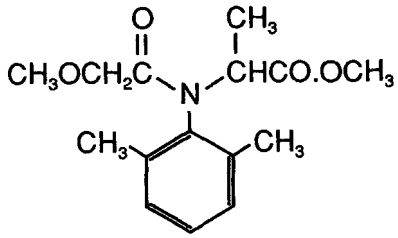
E. Fungicides

The widespread and systematic use of fungicides means that food, air, soil, and water must often be monitored for residues of these toxic chemicals. Many of the IAs that have been developed for the detection of fungicide residues in food could, with minor adaptation, be used to screen environmental samples. Lukens and Williams raised ABs to 2-aminobenzimidazole (2-ABZI), a degradation

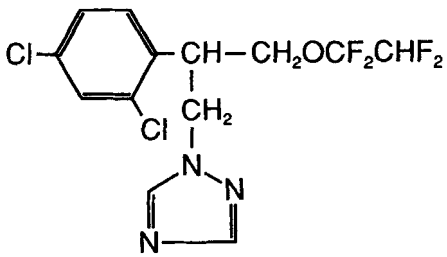
product of benomyl (Figure 16) and other carbamate fungicides.[123] A fluorescent hapten was formed by the conjugation of 2-ABZI to fluorescein isothiocyanate. The ABs cross-reacted weakly with benzimidazole in the FIA. An early attempt to develop an RIA for the analysis of ethylcarbamate in wine failed because of the apparent background contamination of goat sera with anti-ethyl carbamate ABs.[276]



Benomyl
(2-ABZI: a=NH_2, b=NH)



Metalaxyl



Tetraconazole

FIGURE 16. Molecular structures of various fungicides.

Newsome and Shields used RIA to determine residues of benomyl on food crops.[277] Since benomyl breaks down to methyl-2-benzimidazolecarbamate (MBC) during extraction with ethyl acetate, the amount of MBC measured by the RIA reflects the sample's combined benomyl and MBC content. The ABs also bound 2-benzimidazolyl urea and 2-aminobezinimidazole; this should not be a problem since neither compound is a significant metabolite of MBC. Several compounds that were thought likely to occur in association with benomyl did not cross-react in the assay. Newsome and Collins later developed EIAs for benomyl and thiabendazole (TFZ).[278] The coating AGs were synthesized from succinamido derivatives of the haptens (Table 1). The antiserum and analyte were preincubated for 15 (benomyl) or 30 (TFZ) min. TFZ solubilization was aided by 0.1 *N* HCl. The benomyl EIA had a shallower calibration curve but a lower DL than the RIA (Table 6). The benomyl EIA's specificity differed from the RIAs: 2-ABZI did not cross-react in the EIA. The anti-TFZ serum cross-reacted slightly with MBC and 2-benzimidazoleurea. Bushway et al. used Newsome's approach to produce antiserum to MBC; the antiserum was used in a rapid tube-based EIA for MBC (Table 6) that is now marketed by IMS, Inc. and Millipore.[279]

Analytical efficiency was improved by a factor of 4.5 when an EIA was used to screen food samples for metalaxyl (Figure 16) residues.[280] The immunogen and coating AG were prepared by means of water-soluble CDI and mixed anhydride reactions, respectively (Table 1). The sample and antiserum were preincubated for 30 min. The ABs cross-reacted with the following compounds: metolachlor, diethatyl ethyl, furalaxyl, and alachlor. Such broad specificity indicates that the assay would be mainly useful as a screening tool.

An EIA also has been reported for the detection of triadimefon in foods.[281] The coating AG for this assay was prepared from the derivatized hapten (Table 1) using a mixed anhydride reaction. The assay, which was similar in design to the EIA for metalaxyl, also detected triadimenol, a metabolite of triadimefon.

Iprodione also should be amenable to analysis by IA.[282] However, the only reported assay used ABs that had poor specificity; probably because they were directed against the heterocyclic portion of iprodione. Several rearrangement and hydrolysis products of iprodione interfered in the assay. The fungicides Vinclosolin and procymidone were 3.5 and 10 times more reactive than iprodione in the assay; however, those fungicides are not licensed for use in Canada, where it was intended to use the assay.

TABLE 6
Immunoassays for Fungicides

Analyte	ABs	Format	Label	Sep. sys.	Range (dl) buffer	Range (dl) matrix	Ref.
2-Aminoben-zinimidazole	P	FIA	Fluorescein-labeled hapten	n.a.[a]	100 pg/ml–160 ng/ml (100 pg/ml)		123
Benomyl and methyl 2-benz-imidazolecarbamate	P	RIA	[2-^{14}C]benzimidazolecarbamate (15 mCi/mmol)	DCC	1 ng–30 ng (2 ng/ml); I_{50}: 7 ng	Variety of fruit: 1–10 ppm	277, 278
(1) Benomyl (2) Thiabendazole	P	EIA	Labeled DAB	IH	(1) 0.06–1 ng/ml (0.12 ng/ml); I_{50}:1.4 ng/ml (2) 0.03–0.5 ng/ml; I_{50}: 0.21 ng/ml	Variety of produce 0.1–2 ppm [(1): 0.35 ppm; (2) 0.03 ppm)]	278
MBC	P	EIA	Labeled hapten	IAB	1-26 ng/g (100–2600 pg/tube)	(10 ppb juices; 30 ppb concentrates)	279
MBC	P	EIA	Labeled hapten	IAB	0.69–22 ng/ml (0.5 ng/ml) I_{50}: 4 ng/ml	Blueberry extract: 0.69–22 ppb (18 ppb)	334
Metalaxyl	P	EIA	Labeled DAB	IH[b]	50 pg/ml–1 ng/ml (63 pg/ml); I_{50}: 0.5 ng/ml	Variety of produce 0.1–2 ppm (0.1 ppm)	280
Triadimefon	P	EIA	Labeled DAB	IH	1–16 ng/ml (1 ng/ml);[c]	Variety of produce 0.5–2 ppm	281
Triazoles	P	EIA	Labeled DAB	IH	(a)DTP 10–1200 ng/ml (30 ng/ml);[c] I_{50}:1 µg/ml		286
(b)tetraconazole 9–850 ng/ml					(6ng/ml);I_{50}: 0.15 µg/ml		
Iprodione	P	EIA	Labeled DAB	IH	0.2–20 ng/ml (0.2 ng/ml); I_{50}: 3.8 ng/ml	Variety of produce 0.1–1 ppm (0.03 ppm, practical 0.1 ppm)	282
Fenpropimorph	P	EIA	Labeled hapten	IDAB[d]	20 pg/ml–2 ng/ml (13 pg/ml); I_{50}: 300 pg/ml (6 pg/well)		283
Blasticidin S	P	EIA	Labeled hapten	DAB	100 pg/tube–10 ng/tube (100 pg/tube); I_{50}: 1 ng/tube		287

[a] Not available.
[b] Immobilized hapten
[c] Estimated from lower limit of calibration curve by present author.
[d] Immobilized double antibody technique

Fenpropimorph is a difficult to analyze fungicide that requires enrichment and derivatization; it is thus an ideal candidate for analysis by IA. Jung et al.[283] used a sensitive variant of the direct competitive heterogeneous EIA[284, 285] for the detection of fenpropimorph and its metabolite fenpropimorph acid. The microtiter wells were first coated with affinity-purified second AB. Analyte, enzyme-labeled hapten, and antiserum were then added to the appropriate wells. After overnight incubation, the wells were washed and the enzyme reaction was developed. The performance of sera from four rabbits varied markedly, which again demonstrates the benefit of using multiple test animals. The selected serum had an optimal dilution of $1:2 \times 10^5$, which is considerably higher than normal and suggests that the IDAB format can help to conserve a finite reagent. The IDAB assay format improves assay precision by reducing color variability between wells. Tridemorph was the only related compound that cross-reacted (2%) in the assay; the antibodies could distinguish between the *cis*- and *trans*-stereoisomers of fenpropimorph.

Researchers in Italy have reported the development of an EIA for the triazole fungicides.[286] The ABs were raised against 2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazoyl-1-yl)propanol (DTP) (Table 1) and purified by chromatography on protein A. The serum cross-reacted strongly with the commercial triazole fungicides tetraconazole (Figure 16) and penconazole. Other fungicides that contain either the imidazole, pyrimidine, or pyridine groups did not cross-react strongly. Further research is under way into the effect of alternate coating haptens on the assay's sensitivity.

The antibiotic Blasticidin S (BLS), used as a fungicide in rice culture, is currently analyzed by means of bioassays. Kitagawa et al.[287] raised anti-BLS sera in rabbits that were immunized using a BLS-protein conjugate that was prepared using N-(*m*-maleimidobenzoyloxy)succinimide (MBS, available from Pierce) as a cross-linker (Table 1). The ABs were highly specific for BLS and showed little cross-reactivity for other commonly used antibiotics.

F. Miscellaneous Analytes

IAs have been developed for a variety of other chemicals that are either actual or potential environmental contaminants. An indirect EIA was found useful for the analysis of 2-methylisoborneol (MIB), a metabolite of algae and actinomycetes that is a common cause of off flavors in water.[288] Camphor, a related compound, was used as a surrogate hapten (Table 1). The antisera were raised in goats and the ABs were isolated by chromatography on protein-G. Camphor-ovalbumin (OVA) was used as the coating AG. Changes to the coating AG and enzyme reaction failed to improve the assay's moderate sensitivity (5 to 1.25 μg/ml). The assay, which was selective for camphor, camphorquinone, MIB, borneol and isoborneol, and 2-methyl-2-bornene, would be more useful if its sensitivity were enhanced. Several approaches were subsequently used in an effort to improve the assay's sensitivity.[289] A spacer arm was inserted between the camphor hapten and the carrier protein, which resulted in a higher titer antiserum. The improved titer, however, had no effect on assay sensitivity. A direct competitive EIA format that used an alkaline phosphatase label and an enzyme amplification system also failed to lower the assay's I_{50} value. Different blocking agents, microtiter plates, and variations in shaking and incubation times also were unsuccessful. Further efforts to improve the assay's performance would probably have to focus on the generation of higher-affinity ABs.

Wie and Hammock reported a useful EIA for the determination of the widely used Triton X series of nonionic detergents.[290] Inasmuch as these surfactants are nonvolatile and unreactive, they were considered suitable for analysis by IA. The assay readily detected all members of the Triton X series, and had a DL of about 1 ng/ml of Triton X-100. The assay was about 20 to 100 times less sensitive for the Triton N series of detergents, although they were still detectable. Other neutral and ionic detergents did not cross-react.

IAs can play an important role in monitoring the presence of pharmaceutical contaminants in the aquatic environment.[22,291] Aherne et al.[292] used clinical assays to screen a variety of water samples for the presence of natural and synthetic steroids and the anti-cancer drug methotrexate. The assay detection limits were 5 to 10 ppt in preconcentrated water. Norethisterone (17 ppt) and progesterone (6 ppt) were detected in river water, and methotrexate (1 ppb) was detected in hospital effluent. Periodic testing of the waters would assure that the risk to the public's health remains low.

Many assays that have been developed for the detection of antibiotics in physiological fluids, food, and farm animals [293-297] also could be adapted for use in the analysis of water and biota. Antibodies and associated assays have been reported for aflatoxins,[298,299] ochratoxin A,[79,300,301] trichothecenes, and zearalenone[131,302-305] among other mycotoxins.[72] An innovative EIA has been recently developed for the detection of mercuric ions in water.[306] The assay is based on an MAB that binds specifically to immobilized mercuric ions. The assay has a working range of 0.5 to 10 ppb and proved as sensitive as cold-vapor atomic absorption spectroscopy. Two hybridoma clones that could distinguish between BSA-glutathione and BSA-glutathione-$HgCl_2$ were selected. The microtiter plate wells were coated with BSA-glutathione. Water samples were added to the coated wells and incubated for 30 min. Any mercury in the sample becomes bound to the glutathione where it in turn can be bound by the ABs. The remainder of the assay follows a conventional indirect EIA format. Other metal ions do not interfere in the assay. Unfortunately, interference from chloride ions at concentrations of at least 1 m*M* could limit the assay's use for the analysis of seawater.

III. PAB OR MAB TECHNOLOGY?

Both poly- and monoclonal ABs have been used to develop effective environmental IAs. Polyclonal technology has proven to be a cost-effective source of ABs to many pollutants; about 80% of the published environmental IAs have used PABs. Opinions differ as to which clonal type should be favored for future methods. One of the main advantages of hybridoma technology is the ability to select clones that secrete ABs of the desired specificity and sensitivity. Once selected, these clones can be used to produce a virtually unlimited supply of MABs.[135] AB supply will become a key factor in the case of environmental IAs that become accepted for widespread use. MABs, however, are costly to develop, and the expense may not always be justified for environmental applications[210] — at least during the initial investigations of a method's usefulness. For the present, finite resources might be more effectively used in the validation and implementation of some promising IAs in real world applications, since the main challenge is to gain wider acceptance of IAs as legitimate analytical tools. For the future, there is little doubt that regulatory and legal pressures will demand carefully standardized and reproducible methods and that this will foster the wider use of MAB technology,[210] which can be used to standardize the primary ABs.[16,4,40,77] Eventually, MABs will probably become the key component of IAs that are approved and standardized for the detection of environmental contaminants.[130,154] If, as expected, the cost of producing MABs declines as the technology matures, MABs may well become the clonal type of choice, especially for commercial assay kits.[85,307]

A. Assay Specificity and AB Type

Chief among the perceived shortcomings of polyclonal sera are poor selectivity and a variable immune response. Owing to their broad specificity, PABs often cross-react with molecules that are closely related to the target analyte. Cross-reacting compounds that are themselves pollutants or are unlikely to occur in the matrix of interest are not a serious concern. Moreover, it is often desirable to detect a range of related contaminants or breakdown products; for example, the breakdown products of many pesticides are themselves highly toxic. Furthermore, the presence of the degradation products of a pesticide may reveal prior contamination with the parent compound. Thus, sera of broad specificity are suited to many screening tasks. If an application requires high selectivity, several steps can be taken to improve serum specificity. Sera and clones should be screened for acceptable selectivity at an early stage in the assay development process. If this initial screen is unsuccessful, the cross-reacting ABs can be removed by affinity chromatography. Often the immunogen can be redesigned so as to narrow the induced serum's selectivity. The systematic design and evaluation of immunogens and coating AGs has produced many sensitive and selective PAB-based assays; often, the reagents can be adjusted so that either a class of compounds or a single analyte are detected.[134] Frequently, adjustments to the assay's kinetic and thermodynamic parameters, such as assay temperature and duration of incubation, can help to minimize unwanted cross-reactions. The reader is referred to a recent review article that deals with the latter topic in depth.[190] Serum variability can be a frustrating problem. Sera

from different animals can have different specificities, even when raised against the same immunogen; even serial bleeds from the same animal can vary.[42] The use of large host animals such as goats or horses or the pooling of sera can provide large pools of homogeneous sera.[17]

The present report contains several examples of assay specificity problems that were corrected through the use of alternative immunogens. For example, a serum that was raised against a 2,4-D hapten that had been conjugated to carrier protein through the 5-position on the aromatic ring was more sensitive to 2,4,5-T than 2,4-D (CR% = 19).[170,171] Serum specificity was reversed by the conjugation of 2,4-D through the acetic acid moiety.[171,172,174] Sera raised against atrazine haptens that were conjugated through the 2-Cl position to carrier protein (Table 1)[176,194] tended to cross-react with a wide range of triazine herbicides. By coupling atrazine through the ethylamino position, Dunbar et al.[177] and later Wittmann and Hock[183] and Wust and Hock[28] were able to narrow the assay's specificity. Anti-atrazine MABs that were raised against an immunogen similar to Dunbar's had a comparable specificity pattern.[196] A thorough investigation by Harrison et al. confirmed that conjugation position and alkyl substitutions can play an important role in the determination of serum specificity.[199]

For applications that require the differentiation of the target analyte from close structural relatives, MABs, because of their usually narrow selectivity, can have the edge. Hybridoma techniques can be used to solve many stubborn specificity problems. MABs could probably be used to narrow the selectivity of the PAB-based IAs that were developed for the following analytes: molinate,[229] chlordane,[237] endosulfan,[238] metalaxyl,[280] and iprodione[282] — all of which suffered from selectivity problems. The selectivity of MABs, however, is not always superior to the equivalent polyclonal sera. This point is well illustrated by the anti-paraquat MABs and PABs that were generated by Niewola et al.,[205] which had similar specificities, with the exception of the MABs higher affinity for diethylparaquat (CR% = 214 vs. 40).

Hybridoma technology was used to produce ABs that could bind free Soman (MW = 183) after earlier efforts with sera were fruitless.[259] A MAB-based IA for paraoxon was more selective than its PAB equivalent but still cross-reacted with parathion and *p*-aminophenyl paraoxon; the MAB assay also had lower sensitivity.[252, 254]

The anti-dioxin MABs of Vanderlaan et al.[154] were selective for the highly toxic PCDD and PCDF congeners while discriminating against the less toxic lower and higher chlorinated congeners. Anti-PCDD sera, however, could still have a role because of their ability to provide information on a broader range of homologs.[29] A selection of anti-dioxin MABs, each targeted against a different congener group, would be an invaluable tool since it would enable the analyst to select the binder based on analytical requirements.[39]

In the past, MABs have tended to have lower affinities,[39] and thus inferior DLs to rabbit sera.[85] Since sera contain ABs with a variety of affinities, a small number of high-specificity and high-affinity ABs can govern assay performance at low analyte levels.[10] The use of multiple mouse strains and improved clonal selection techniques would favor the selection of high-affinity MAB clones. This strategy probably offers the best prospects of generating high-affinity ABs for low molecular analytes, such as glyphosate or amitrole. Recent innovative assay designs, such as the IDAB format, have been used in highly sensitive MAB-based assays for environmental contaminants (Table 4).

Some caution should be exercised in the interpretation of data from the standard cross-reactivity tests. Cross-reacting compounds that are likely to be encountered in real world samples also should be tested at realistic concentrations that match their expected or known environmental levels. Otherwise, a false sense of security in the assay's specificity can be created. This can happen with analytes that exhibit low cross-reactivity at low concentrations, but cross-react to a much greater extent when present at elevated concentrations. A more thorough method of calculating cross-reactivity that involves measuring the bias due to cross-reactants in the presence of the target analyte has recently been proposed.[388]

IV. ASSAY SENSITIVITY

Ekins defines assay sensitivity, which is primarily related to AB affinity or serum avidity, as the imprecision of measurement of zero dose.[308] It fol-

lows from that definition that an assay's DL is the least amount of analyte that can significantly ($p < 0.05$) inhibit tracer binding.[24] Thus, the DL is directly related to the experimental error associated with analyte-free blanks from which it is commonly deduced. The zero analyte's error can be represented by the standard deviation (SD) of either the B_0 tubes or of the negative control tubes. Alternatively, the concentration-error relationship can be plotted for a heavily replicated (×10) calibration curve; the zero analyte's error is estimated from this plot by extrapolation of the curve to zero dose.[146,308] The nominal working range of an assay is delimited by the calibration curve's linear region. The precision profile (PP) gives a more reliable estimate of an assay's working range since it establishes the concentration limits within which precision is acceptable. The PP may be prepared from an extensively replicated (×10) calibration curve: a PP for the tritium-based RIA for PCDDs is shown in Figure 17 as an example. The amount of analyte that inhibits AB binding by 50% (I_{50}) is often used as a useful indicator of the assay's responsiveness since it establishes the analyte's concentration in the most precise region of the calibration curve.

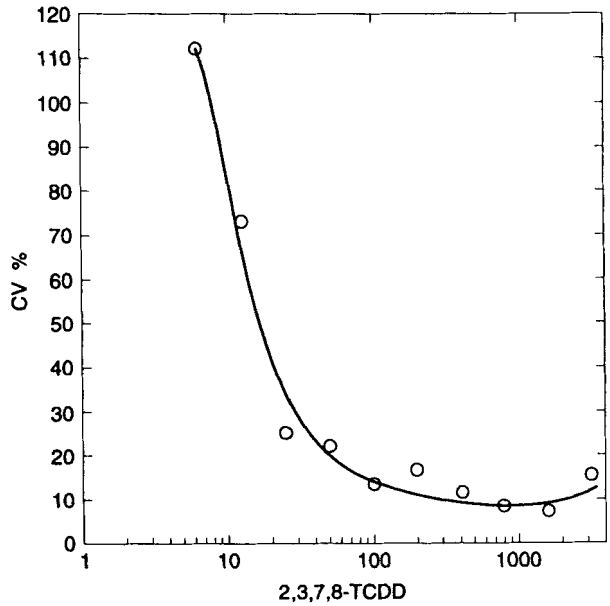


FIGURE 17. Precision profile for the PAB/tritium-based version of the RIA for PCDDs.

A comparison of the sensitivities and DLs of the reviewed methods (Table 3 to 6) is complicated by the variety of techniques that have been used to estimate that parameter. The DL has been most commonly estimated by multiplying the error associated with the measurement of zero analyte by a factor of 1,[205] 2,[196,221,226,229,238,254] 2.5,[203] or 3.[146,150,167,171,186,214,283] The DLs of environmental IAs also have been estimated from the amount of analyte that inhibits tracer binding by two times the CV;[280] multiplication of the mean SD of points within the calibration curve by 3;[282] estimation of the concentration required to cause a specified decrease in tracer binding (10 to 20%);[194,219,220,236,261,266,278,334] estimation of the lower limit of the calibration curves's linear range;[178,229,247,248,258] estimation of nonoverlapping ranges of blank and test replicates;[29,124] differentiation from noise level;[123] estimation of the analyte concentration that corresponds to the value of the 4 parameter logistic at the estimated upper asymptote of the lower confidence limit;[223] estimation of the smallest quantity that can be analyzed in a single analysis with an error of 5%.[185] Some researchers prefer that the DL lie within the calibration curve's linear region, even though lower concentrations may be detectable.[247,248] For reports that do not provide a DL, the tabulated values (Tables 3 to 6) were estimated by the present author from the published calibration curves.[155,160,164,170,174,201,208,215,217,231,240,242,267,272–274,277,281] Many other reports provide a DL but do not describe its derivation.[78,122,125,152,162,163,166,172,176,177,181–183,197,200,216,230,237,249,255,257,260,268,270,287,320]

The practical quantitation limit (PQL) also has been gauged by multiplying the experimental error associated with the zero analyte by a factor of 10.[278,282] When the calibration curve is prepared in the matrix, the DL is equivalent to the assay's quantitation limit. Assay performance characteristics often differ between buffer and matrix systems,[24] and should be determined for each matrix type.

A. HAHs (Summarized in Table 3)

The excellent sensitivity of the RIAs for 4-acetamidobiphenyl and N,N'-diacetylbenzidine (DLs < 10 pg) may have been influenced by the use of succinamido spacer arms in the immunogens. Newsome and Shield's RIA for PCBs also performed well at low analyte levels[160] and warrants a

full evaluation with environmental matrices. Although not exceptionally sensitive, Westinghouse's IA for PCPs is probably adequate for use in surveys of badly contaminated waters. Inasmuch as levels in Canadian waters are usually in the region of a few to several micrograms per liter,[309] solid-phase extraction (SPE) or solvent extraction of samples would be required for general monitoring applications. PCP levels in sediments from industrialized areas of Canada are usually below 50 μg/kg, but levels can be higher close to point sources or spills.[309] The MAB-based EIA for PCDDs appears to have a higher DL than the PAB-based RIA. A controlled comparison should determine whether that difference is attributable to the assay format or the AB's clonal type. Preliminary data for a [^{3}H]-based RIA that uses MABs indicated that the assay's calibration curve is shifted to the left of the PAB's (I_{50} of 210 vs. 350 pg).[310] The [^{3}H]-ligand version of the RIA for PCDDs, particularly in its "low level" variant, was more sensitive than earlier ^{125}I-based assay versions. Since the chlorinated dioxins are routinely determined at ultra-trace levels, the performance of the IAs for PCDDs at low picogram levels will crucially influence their ability to screen extracts of routine samples from the aquatic environment. Nevertheless, the present IAs for PCDDs may prove useful in the screening of samples from badly contaminated areas or in the screening of serum samples from exposed populations.

B. Herbicides (Summarized in Table 4)

Although the performance of Rinder and Fleeker's RIA for 2,4-D and 2,4,5-T varied with each batch of tracer,[170] it was apparently more sensitive than the corresponding EIA;[171] inter-sera differences, however, may have been the main source of variation. The most sensitive IA for 2,4-D was developed by Knopp et al.,[173] whose PAB-based RIA had a DL of 100 pg. Several of the triazine IAs also have excellent sensitivity. Huber's use of ABs that were immobilized on polystyrene spheres facilitated a low DL by permitting the analysis of large sample portions (20 ml).[176] Two recent conventional EIAs from the same laboratory had DLs of 20 ppt and 1 ppt of atrazine.[183,184] The excellent sensitivity of the latter[183] EIAs for atrazine and propazine (Table 4) was partly due to the choice of enzyme tracer: a peroxidase atrazine conjugate that was prepared using a CDI/NHS active ester procedure. This ultra-efficient label permitted the use of higher tracer dilutions than was possible for an alkaline phosphatase-based system with a resultant improvement in assay sensitivity. Wittmann and Hock used an innovative immunization protocol to generate a sensitive serum for deethylatrazine and deisopropylatrazine;[185] the serum was combined with the efficient peroxidase tracer to yield a highly sensitive assay. The sensitivity of IMS's EIA for atrazine was dramatically improved when the slightly less convenient microtiter-strip format, called RES-I-QUANT, was introduced (Ferguson, personal communication). The MAB-based EIAs for atrazine and hydroxyatrazine demonstrate that the advantages of MAB technology need not come at the expense of assay sensitivity. The enhanced luminescence IA for the detection of triazines could detect analytes at a threshold level of 100 ppt, although accuracy was lower in the region of the DL.[187] The MABs to triasulfuron yielded assays with impressively low DLs that lay in the region of 5 pg/ml[226] — which lends support to the theory that assay sensitivity can be expected to improve as MAB production techniques become more refined.

Of two MAB-based assays for paraquat, one was reasonably sensitive but lacked selectivity,[205,311] and the other was highly selective but somewhat insensitive.[208] A MAB-based EIA for picloram was more sensitive than the corresponding PAB-based EIA (× 5) and RIA (× 50),[230] mainly because its calibration curve was steeper and shifted to the left. However, the RIA's sensitivity could probably have been improved if a radioligand of higher specific activity were used. The comparable sensitivities of the paraquat RIAs and the better of the EIAs suggest that neither tracer type is inherently superior.

Several of the IAs for small herbicide molecules such as maleic hydrazide and molinate required a preconcentration step for low-level analyte levels. The mid-curve response of the molinate assay was improved by a factor of 4 to 5 when the IDAB format was used.[78] This format could probably be used to good effect with other analytes. The improved sensitivity is probably a consequence of the reduced levels of AB and tracer required to produce a measurable response: competitive bind-

ing assays are made more sensitive by lowering the levels of AB and tracer. The sensitivity of the EIA for bentazon was improved by derivatization of the bentazon residues: N-methyl and N-ethyl derivatives were most effective.[234]

C. Insecticides (Summarized in Table 5)

The summarized data indicate that the EIA for s-bioallethrin was more sensitive than the corresponding RIA, and that the sensitivities of the RIA and EIAs for parathion were comparable. Optimization of assay design had a profound effect on the performances of EIAs for diflubenzuron and BAY SIR 8514; their DLs were improved by factors of 16 and 10, respectively. The EIAs for diflubenzuron were as sensitive as conventional HPLC and GLC-electron capture detector (ECD) methods.[247] The MAB-based assays for paraoxon and MATP were less sensitive than their PAB equivalents. In the case of MATP, the MAB's superior specificity probably made up for the loss of sensitivity. The magnetic particle-based EIA for aldicarb proved to be significantly more sensitive than the earlier direct EIA that used microtiter plates as the solid phase (Table 5).[220]

D. Fungicides (Summarized in Table 6)

A point of interest is the DL of the benomyl EIA, which was lower than the corresponding RIA's despite the EIA's shallower calibration curve. This emphasizes the inadvisability of using the slope of the assay calibration curve as a measure of assay sensitivity. The exceptional sensitivity of the IDAB-based IA for fenpropimorph may result in part from the improved accessibility of AB binding sites. Unlike conventional immobilization techniques, the IDAB technique positions more of the bound ABs correctly for effective coupling with hapten molecules. The IDAB format also probably causes less denaturation of the ABs compared to direct coating methods. Inasmuch as the second ABs are used in large excess, virtually all of the primary ABs added to each well are bound to the immobilized DABs, which helps to reduce inter-well variability and conserve a valuable reagent.[78,283]

E. Criteria for Sensitive Assays

Assays with I_{50} responses that are close to 1 ng/ml in the final assay buffer have been categorized as highly sensitive;[130] an assay with an I_{50} in the 40- to 100-ng/ml range would be considered to have low sensitivity. By these criteria, several of the assays for HAH-type compounds (Table 3) are sensitive;[29(triton version),124,155(0.25%Cutscum version),150,160,162,163,167] several are of intermediate sensitivity,[29(GC5 serum, cutscum version),125,153,155] and one has low sensitivity.[166] The assays described in Tables 4 to 6 could be similarly classified. One must be aware, however, that assays such as Hunter and Lenz's IA for paraoxon[252] (Table 5), which had a high I_{50} value but a low DL (28 pg/ml), could be misclassified by this scheme.

Most of the reviewed assays are adequately sensitive for environmental screening tasks. In cases where the analyte must be quantified close to the statistical DL, it may be necessary to preconcentrate the sample, prepare a detailed calibration curve, or mathematically extend the curve's linear range.[229] A simple preconcentration step such as SPE or solvent extraction can dramatically improve an IA's PQL, although the improved sensitivity is often unnecessary for screening applications. SPE is particularly convenient for water-borne analytes. Several steps can be taken during assay development to help improve the sensitivity of the final product. These include the use of a spacer arm in the immunogen, which often improves serum avidity by enhancing the hapten's recognition by the host's immune system. Different linkage arms should be used in the immunogen and tracer (or coating AG): heterologous assay systems are frequently more sensitive than their homologous equivalents. The IgG component of the serum can be purified by IAC: affinity-purified ABs tend to be more sensitive, though less stable, than whole sera.

Although the sensitivity of competitive IAs is primarily related to AB affinity or serum avidity, fine tuning of the assay format and mechanics can help improve many assays. For instance, a preincubation of ABs and analyte may help improve the sensitivity of some assays. The excellent sensitivity of the polystyrene sphere version of the triazine IAs[182] and the success of the IDAB format[283] show that innovative assay design can push

an assay's performance to lower limits. The ultrasensitive EIAs that have been originated by Ishikawa et al.[68] use a noncompetitive EIA format that is combined with fluorometric detection of the enzyme's reaction products in a design that should be adaptable for many environmental analytes. Phenomenal DLs in the milli- to attamole range have been reported for these assay systems. A competitive version of this EIA format that was based on the measurement of hapten-occupied binding sites also was remarkably sensitive (DL of 0.1×10^{-15} *M* for thyroxine). Fluorogenic enzyme substrates have also been used to dramatically lower the DLs of EIAs for other analytes of clinical interest.[389] This approach could prove useful in enhancing the performance of EIAs for the detection of ultra-trace environmental analytes such as the HAHs. EIA DLs can also be lowered through the use of signal amplification systems such as biotin-avidin auxiliary labels.[297] In essence, the biotin-avidin system increases the number of enzyme molecules bound to each AG-IgG complex; this allows a reduction in the amount of primary AB that is used in the assay, thus lowering the DL.[16,73,312,389]

V. SAMPLE PREPARATION

The exceptional sensitivity and selectivity of the IA technique often permit a reduction in sample preparation compared to conventional methods.[4,85] Sample preparation can frequently be eliminated in the case of water samples and physiological fluids. IAs have been used to detect the contaminants listed in Table 7 in crude liquid matrices. For many nonaqueous matrices, however, there is a point beyond which there is a trade-off between reduced enrichment and assay sensitivity. IAs, as aqueous

TABLE 7
Use of IAs to Detect Contaminants in Aqueous Matrices Without Clean-Up

Analyte	Matrix	Observations	Ref.
2,4-D	River water, urine, serum, plasma	Sensitivity loss in plasma	172, 174, 346
Picloram	River water, urine		174, 230
Molinate	Water	ppm levels	229, 331, 320
Hydroxyatrazine, atrazine	Water		194, 196, 184, 199, 178, 183, 328, 200, 28
Terbutryn	River water		181
Paraoxon	Serum	Slightly reduced sensitivity	252, 253
Parathion	Serum	Reduced sensitivity	249
Paraquat	Plasma and urine		203, 332
Paraquat	Milk		212
Fenpropimorph	Tap water and soil percolation water		283
s-Bioallethrin	Milk	Reduced sensitivity	243
Diflubenzuron			
BAY SIR 8514	Stagnant water		247
Alachlor	Water	Some matrix effects	217, 219, 328
Diclofop-methyl	Serum and urine		214
PCP	Groundwater		166
Deethylatrazine, deisopropylatrazine	Water, groundwater	No matrix effects	185
Paraoxon	Blood		333
Soman	Water, serum, milk	Reduced sensitivity	262
Aldicarb	Stream water		268, 220
MBC	Juices	Slight matrix effects	279
Carbofuran	Well water		328
Paraquat	Serum	TritonX-100 and sodium dodecyl sulphate used to limit NSB	213
TNT	Tap water		167
4-Nitrophenol	Environmental water		169

phase tests, are generally easier to apply to hydrophilic rather than lipophilic molecules. Polar analytes tend to be located in a sample's aqueous phase where they can either be directly bound by the ABs or from which they can be readily extracted. Nonpolar analytes, on the other hand, are usually present in the lipid or oil fraction of environmental samples from which they must first be extracted. Once extracted, the nonpolar analytes can be solubilized with the aid of nonionic detergents, protein solutions, or small amounts of polar solvent. The ABs draw the analyte from the detergent micelles into the binding site by a process of mass action.[39] The following polar solvents have been used to aid the solubilization of analytes in environmental IAs: DMSO,[147,160,234,249,282,313] acetonitrile (ACN),[229,234,240,247,248,270,320] dimethylformamide (DMF),[235] methanol,[164,200,234,237,262,266,270,271,280,281,320] ethanol,[249,262,267,286,288] propylene glycol,[229,320] and dioxane.[256] Solubilization agents should be carefully evaluated for each assay system because of the unique nature of AB-AG reactions. To illustrate this point, a variety of AB and coating AG systems that were evaluated for the bentazon IA were observed to have strikingly different tolerances for ACN (10 to 50%).[234] Also, the EIA for methoprene was about three times more sensitive when the calibration curve was prepared in MeOH than in ACN. The harmful effects of ACN were offset by the inclusion of 0.5% BSA in the diluent.[270,271]

The need to separate lipophilic analytes from oily matrices is a major impediment to the routine use of IAs for the detection of such compounds. If a low DL is to be achieved, lipophilic analytes (such as dioxins) must be thoroughly separated from residual lipids. For that reason, some experts suggest that highly lipophilic compounds may not be ideal target molecules for analysis by IA since many of the technique's cost and speed advantages are lost during the clean-up process.[129] On the other hand, the high cost of analysis for those compounds ($1500 to $2000 for PCDDs) is a strong stimulus for the development of screening methods. The amount of sample preparation could probably be reduced for analytes and samples that need not be analyzed at ultra-trace levels. Even for analytes such as dioxins, which must often be extensively enriched,[147] IA could reduce the number of samples that must be confirmed by high-resolution GC-MS, thus lowering analytical costs and helping to reduce a lab's capital burden.

Environmental matrices are more variable and interference prone than is common in clinical applications. There are three main types of interference: compounds that cross-react (positive interferences), matrix components that interfere with the AG-AB reaction (negative interferences), and matrix components that interfere with the assay detection system. Sample preparation protocols are designed to eliminate or minimize those interferences and they should be matched to the assay system, the matrix, and the desired level of sensitivity.

Acid digestion of extracted lipids followed by chromatography on basic alumina was used to enrich PCDDs from liver, adipose tissue, and sandy soil samples that were screened by RIA.[29,144] From 3- to 50-mg equivalents of starting adipose tissue was analyzed per assay tube. Some negative interferences that were found to limit assay sensitivity could probably be removed by an improved clean-up method. A similar clean-up protocol was used to prepare Aroclor, liver, and adipose samples that were contaminated with PCDFs for analysis by RIA.[124] Low-level positive interferences from the Aroclor and tissue samples adversely affected the RIA's sensitivity.

A minimized clean-up was used to prepare a set of PCDD-contaminated industrial and soil samples for screening by EIA; the samples were chromatographed on activated carbon and then passed through a combination column that contained acid silica and $AgNO_3$-treated silica.[155] The reduction in the number of clean-up steps enhanced the IA's value as a screening test and was designed to remove hydrophobic matrix material that might overwhelm the detergent-based solubilization system. The reduced clean-up was adequate for the high analyte levels in the chemical and oil samples that were tested. Extracts of a sandy soil that were similarly prepared were analyzable by EIA; however, extracts of other soils interfered with the EIA.

The amount of clean-up used to prepare a Lake Trout extract was found to influence the performance of the RIA for PCDDs.[147] The assay's capacity was related to the degree of clean-up and the choice of solubilization agent. The presence of residual interferences meant that the assay's sensitivity could not be improved beyond a certain point by simply increasing the sample size. Higher than op-

timal increases in the sample size narrowed the assay's working range, adversely affected the dose response, and raised the DL. A more rigorous clean-up may help to lower the assay's quantitation limit by permitting a further increase in the amount of extract that can be added to the assay tubes. Other matrices, such as water, may not require so extensive a clean-up, or, if heavily contaminated, may not demand as low a DL, in which case the sample preparation could be reduced.

Newsome and Shields passed solvent extracts of PCBs from blood and milk through a column of neutral alumina prior to analysis by RIA.[160] Despite slight analyte losses from the spiked matrices, this minimal clean-up sufficed. ACN/water extracts of permethrin-contaminated beef were effectively cleaned up by partitioning against hexane and purification on alumina before analysis by EIA.[240] The assay's response was linear from 50 to 500 ppb in the presence of extract. Clean-up by SPE on C18 cartridges was required before extracts of wheat or barley could be screened for imazamethabenz content by EIA.[232]

Wie and Hammock did not observe matrix interferences when using EIA to analyze diflubenzuron and BAY SIR 8514 in stagnant or WHO synthetic water.[247] When whole milk was analyzed, however, it was necessary to extract the samples with EtAc and use a multistep clean-up.[248] Calibration curves prepared in the final extract indicated that the extract was interference free.

IAs can frequently be used to detect more polar analytes in solid matrices after a simple extraction with solvent or acid and resuspension of the extract in buffer.[127] This point is borne out by the contaminants listed in Table 8, which were analyzed by IA in crude extracts of a variety of matrices. Goh et al.[315] studied the effects of the extraction solvent on the tube version of IMS's EIA for atrazine and recommended that for field applications the residual solvent should be diluted to at least the maximum tolerable level; for laboratory applications, the solvent can be easily removed. The ability of four solvents to extract atrazine from soil was found to depend on the concentration of atrazine.[316] A methanol-water mixture was best suited to the EIA. The effect of residual solvents needs to be established for individual assay systems because assay design is known to affect susceptibility to residual solvent effects.[199] IMS's microtiter plate-based EIA for atra-

TABLE 8
Use of IAs to Detect Contaminants in Crude Matrix Extracts

Analyte	Matrix	Observations	Ref.
Benomyl	Food, produce		277, 278
Metalaxyl	Food, produce		280
Triadimefon	Food, produce		281
Iprodione	Food, produce		282
Chlordane	Soil		237
Parathion	Produce	Reduced sensitivity	249
Picloram	Soil		230
Hydroxyatrazine atrazine	Soil		194, 196
Atrazine	Soil	Some positive interferences	315
Molinate	Soil	Positive interferences	320
Clomazone	Soil	Some negative interferences	215, 216
Paraquat	Soil		206, 311
Paraquat	Beef, potatoes		212
Chlorsulfuron	Alkaline soil extracts	Some matrix interferences	225
Diclofop-methyl	Soil and biota		214
Metolachlor	Soil	Slight interference in direct EIA	221
MBC	Berries	Slight positive interference (3 ppb)	334
Methoprene	Wheat, tobacco	Enhanced performance in wheat matrix	270, 271
Urea herbicides	Potato, wheat	Matrix interferences with Linuron assay	227
Triasulfuron	Soil	Blank matrix effects, removed by clean-up	226
Atrazine, deethyl atrazine	Soil		186
Fenitrothion, CPM, PM	Grain		266

zine also was interfaced by Stearman and Adams for the extraction of soil.[317] Their data suggested that vortex mixing at room temperature in ACN:H_2O (9:1) followed by overnight incubation was more effective than soxhlet extraction in ACN:H_2O or vortex extraction in ammonium acetate. Each of the solvent systems affected the EIA when the solvents level was greater than 5%. The ACN:water mixture proved superior to MeOH or ammonium acetate. In another study, a very simple aqueous extraction system method that recovered 1/3 to 2/3 of the MeOH extractable analytes was used to extract atrazine and deethylatrazine from soil samples for screening by EIAs. When EIA was used to measure atrazine in milk samples, it was necessary to prepare the atrazine calibration curves in milk of a similar lipid composition to the samples in order to counteract matrix effects.[318] It was postulated that lipid in the high-fat milks solubilized the atrazine and rendered it inaccessible to the ABs.

The pH and ionic environment of samples and standards should be similar, which may necessitate adjustments to the assay and diluent buffers,[212,234] so as to prevent curve shifting effects. Concentrated PBS (10×) can be used to equalize ionic effects in water[166,229] and juice samples.[279] IMS's EIA for triazines has been reported to be resistant to interferences caused by humic substances.[319]

Residual interferences can be revealed by the preparation of an assay calibration curve in the presence of the matrix.[4] In the absence of matrix interferences, the curve prepared in matrix should parallel the control curve. Minor interferences may cause a slight shift in the curve's location, which can reduce assay sensitivity without affecting parallelism. In the absence of serious matrix interferences, the addition of a spike to the sample extract should cause additive inhibition. The routine use of matrix blanks is a good safeguard against residual matrix interferences.[4] IAC techniques can be used to conveniently prepare matrix blanks.[297] In most cases, slight matrix-induced biases should not limit an assay's usefulness.

SPE, usually using C18 or C8 bonded silica, is a convenient way to enrich trace analytes from aqueous samples. If the target compound can be eluted from the SPE column by a water-miscible solvent such as methanol or ACN, there should be no need for a solvent removal step.[320] Should a solvent exchange be necessary, a small volume of keeper solvent, such as propylene glycol, can prevent losses of volatile analytes.[78] SPE methods are easily automated, which makes them ideal for use with large sample sets. SPE has been used to prepare the following water-borne contaminants for screening by IA: 2,4-D and 2,4,5-T,[170,171] molinate,[320] PCP,[166] *B. thuringiensis israelensis* d-endotoxin,[274] bentazon,[234] benzo-a-pyrene,[164] S-triazines,[319] and cyanazine.[137]

Supercritical fluid extraction (SFE) was used to extract 4-nitrophenol and parathion residues from soil samples for subsequent screening by EIA.[314] An oxidant was used to convert the extracted parathion to nitrophenol so as to render it detectable by the anti-4-nitrophenol ABs (Tables 1 and 3).

Russell et al. were the first to report the ability of ABs to bind AGs in anhydrous organic solvents.[75] MABs raised against 4-aminobiphenyl were immobilized on glass beads for the experiments. The ABs functioned better in hydrophilic solvents. Recently, Stocklein et al.[321] studied the ability of two immobilized PAB preparations (C14 and C193) to bind triazine herbicides in organic solvents. Binding to C14 appeared to be positively influenced by solvent polarity, whereas binding to C193 was more dependent on atrazine's solubility in the solvent. The specificity of the ABs for atrazine was higher in toluene than in buffer. Thus, it may be possible to screen triazines after elution from SPE columns without the need for a change of solvents. Anti-progesterone ABs also have been observed to retain their activity in hexane, although calibration curves were shifted to the right and the reaction rate was slowed somewhat.[322] ABs are thought to retain their activity in solvent because of a residual shell of surrounding water. The nonconjugated AB loses activity in the solvents. Anti-aldrin ABs conjugated to a carbohydrate polymer were reported to retain 96, 60, and 57% of their binding affinities in ACN, MeOH, and 2-propanol, respectively.[323] The ability of ABs to function in organic solvents may find application in the immunoassay of hydrophobic compounds such as HAHs.

VI. VALIDATION AND ROUTINE APPLICATIONS

One of the most informative ways to validate IA performance is to make a critical comparison of

its performance with that of an independent control method.[77,78,85] The data in Figure 18 illustrate the results of such a validation exercise; in this case, EIA and GC/MS were used to determine alachlor in water samples. Samples from a variety of locations should be used in any validation exercise because of the inherent variability of environmental matrices; assay performances should be rigorously confirmed and validated for each matrix type. The samples can be subdivided and fortified in order to test for additivity of the dose response.[155] The key assay performance and reliability parameters such as sensitivity and precision need to be established for each intended matrix. There is a recognized need for a generally acceptable protocol for the validation of assays,[78,85] so that assay developers and end-users can have equal confidence. A recent interlaboratory comparison of atrazine EIAs that was held in Germany was a step in the right direction.[324] Several federal government agencies in the U.S. as well as the Association of Official Analytical Chemists (AOAC) and the IUPAC are reported to be developing guidelines for the evaluation of EIA kits.[133] It has been suggested that screening techniques should not have to meet the same rigid requirements of quantitative methods.[11] Kaufman and Clower[133] recently published an informative account of the review and approval process that has been proposed by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture.[325] FSIS wisely recommends that particular attention be paid to points slightly above or below the cut-off value in validation experiments. Applicants would have to assure a 1-year supply of key reagents. The cornerstone of the proposed approval process will be successful assay performance in a supervised collaborative study. The EPA is reported to be correctly placing the onus for documentation and optimization of an assay on the developer and making a requirement that adequate pools of key reagents, such as ABs, be available before an assay can be approved.[133] A method's final acceptance will depend on its performance in a collaborative study. The AOAC has published guidelines for collaborative studies that are intended to validate analytical methods. Those guidelines could be readily adapted by regulatory organizations as the basis for an approval process.[387] Once approved for routine use, it is critical that a kit's performance be assured by the manufacturer in the long term.[388]

A. HAHs and Related Compounds

Johnson et al.[162,163] validated their RIAs for the detection of biphenyl compounds by demonstrating

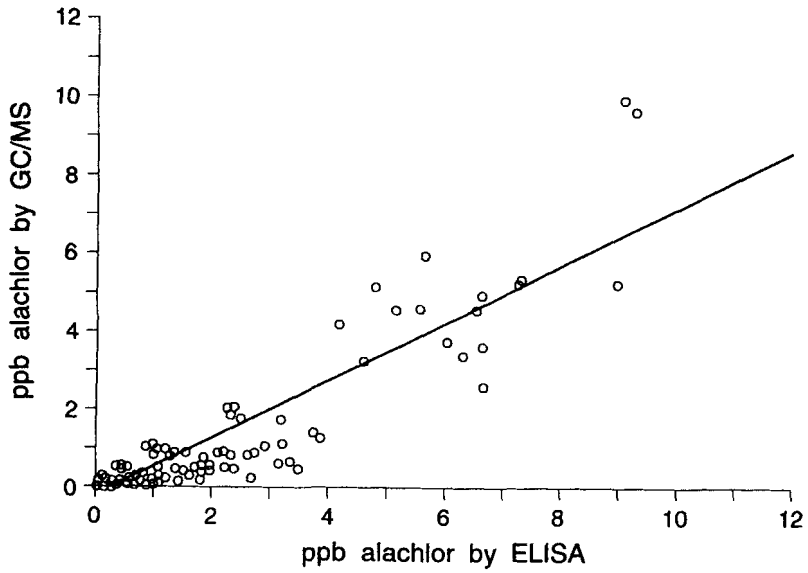


FIGURE 18. Validation of EIA for alachlor by comparison with GC/MS (R = 0.84). (Reprinted with permission from Feng et al., *J. Agric. Food Chem.* **1990,** *38,*159–163; American Chemical Society.)

that the assay calibration curves were parallel to curves prepared in fortified urine samples ($R \geq 0.99$). Newsome and Shields compared the ability of GLC and RIA to recover PCBs from fortified milk (20 to 80 ppb) and human blood (2 to 16 ppb).[160] Both methods compared well ($R^2 = 0.96$ for milk and 0.99 for blood), although the average recovery of analyte was inferior for the RIA (74 vs. 99% for milk and 79 vs. 96% for blood). Westinghouse validated their EIA for PCP by comparing the ability of GC and EIA to recover analyte from fortified ground water and surface water. Both methods yielded similar results for both crude water samples and SPE extracted samples, which indicated the absence of serious matrix effects.[166] The EPA have evaluated the ability of the EIA for PCP to screen drinking water, ground water, and surface water.[392] Spiked and unspiked samples were analyzed at the Westinghouse and EPA laboratories. The relative standard deviations for the EIA tests was 10 to 12%, which compared well with the value of 10% for the confirmatory GC method. No false-negative results were generated in either laboratory and the false-positive rate was less than 10%. ANOVA failed to uncover any significant differences between EIA and GC methods or between the IA performance at the two labs, apart from a difference between the EIA results for unconcentrated water samples. The variability of the EIA results was higher when unconcentrated water was analyzed. Although the EIA yielded more variable data than that produced by the GC method, the EIA's other characteristics support its potential as a screening technique.

Albro et al. assessed the ability of RIA to detect PCDDs in liver and adipose tissue from PCDD-contaminated monkeys.[29] There was good agreement between the RIA and the GC/MS and GC/EC techniques. Afghan et al. subsequently used RIA and GC-MS to detect 2,3,7,8-TCDD in a variety of fish extracts.[326] Low-level false-positive results were probably caused by matrix interferences or the presence of congeners other than 2,3,7,8-TCDD. A double blind evaluation of the RIA that used minimally enriched extracts of human adipose tissue also was undertaken.[144] Each sample (60 mg/tube) was independently analyzed by GC/MS and RIA (3×). The number of false-positive samples (5.9%) and the 57% false-negative results at the lowest spike level (18 pg) indicated that additional clean-up was needed. The RIA had a reliable sensitivity of 100 pg ($p < 0.05$) per tube: 100 pg in a 60-mg sample is 1.7 ppb. The performance of the RIA for PCDDs also was evaluated using fortified Lake Trout extracts.[147] Samples were deemed positive if they were statistically distinguishable from a matrix control using a one-sided t test. The lowest concentration detected was 67 ppt of 2,3,7,8-TCDD in 300-mg equivalents of fish ($p < 0.05$).

The MAB-based EIA for PCDDs was validated using a variety of industrial and soil samples that contained between <1 to 1750 ppb of the most toxic PCDDs/Fs.[155] The enriched extracts were subdivided and analyzed by EIA, confirmed by GC/MS, and tested for an additive response. The GC/MS and EIA results were correlated for the total amounts of tetra- and penta-PCDDs and PCDFs. This was considered a satisfactory result since the two methods do not measure exactly the same congeners. With the exception of some of the soil samples, the EIA responses were additive. The applicability of the RIA for PCDFs was demonstrated by the measurement of analyte in rat and monkey tissues;[124] GC/MS and liquid scintillation techniques were used as control methods. The EIA for benzo-a-pyrene underestimated BP metabolite levels by about 70% when a BP reference curve was employed.[164] The accuracy and precision of the EIA for TNT were examined by spiking tap water with the target analyte.[167] The EIA for 4-nitrophenol and monosubstituted-4-nitrophenols was able to detect the target analytes in a variety of environmental waters without evidence of matrix effects.[169]

B. Herbicides

RIA was used to accurately recover 250 to 2500-ppb spikes of picloram from river water and urine.[174] The inter- and intra-assay precision of MAB and PAB versions of a picloram EIA were assessed using calibration standards and fortified samples.[230] The mean assay precision was excellent for the calibration standards (intra-, <7%; inter-, <16%). When fortified plant extracts were analyzed, the intra-assay variability of the MAB assay (CV% = 10 to 29) was found to be much lower than that of the PAB assay (mean CV% > 80). Only the MAB-based assay was able to efficiently recover spikes from fortified water (78%), soil extract (73%), and plant extracts (112%). Despite some systematic in-

terferences, a dose response was established for each matrix. The EIA for chlorsulfuron also was prone to matrix effects, although it was still possible to establish a dose response for soil extracts.[225] The chlorsulfuron assay's PQL compared favorably with both bioassay and HPLC methods. Similar interference problems were encountered when the MAB-based EIA was used to assay triasulfuron in soil extracts.[226] A clean-up step reduced the interferences to acceptable levels. The assay was validated by fortification of various soils over the concentration range of 0.1 to 10 ppb; the mean recovery was 78% and the CV ranged from 3.2 to 26%. Schwalbe et al.[214] used fortified extracts of biological and soil samples to validate their FIA and EIA methods for diclofop-methyl. The IA results correlated well ($R \geq 0.99$) with both a GC and a liquid scintillation control method.

Good agreement and acceptable precision (inter-, 14%; intra-, 6.3%) were observed when a set of hydroxyatrazine spiked soil samples were analyzed by IA and HPLC;[196] the overall variation in recoveries was from 46 to 100%. A set of 19 soils (for hydroxyatrazine) and 28 water samples (for atrazine) were analyzed; apart from some HPLC-related interferences, the methods compared well ($R = 0.91$ for water). The soil extracts contained some interferences that were probably caused by residual matrix components or cross-reacting triazenes. IMS's tube-based EIA for atrazine had good precision over its working range when used to analyze a variety of water (CV% = 4.1 to 23.8) and soil (CV% = 6.8 to 20.3) samples.[194] This rugged assay also compared well with a reference HPLC method when used to analyze a variety of fortified water and soil samples. The few discrepancies observed were probably caused by cross-reacting triazenes or their degradation products. Bushway et al. used a set of water samples from Czechoslovakia to compare the performance of the tube-based IA for atrazine with a reference HPLC technique.[327] The water samples contained a wide range of atrazine residues (0.2 to 12700 ng/ml). The methods gave consistent results that correlated well ($R = 0.998$; log-log comparison). The EIA's repeatability was assessed using a set of fortified water samples. The samples were analyzed once on each of 6 days. The CV% ranged from 5.7 to 10.9 over the fortification range of 0.25 to 10 ng/ml; this is more than satisfactory for a residue method. The method's precision also was examined using nine water samples (CV% = 5.9 to 26.2). The EIA results showed a high bias, which may have been caused by cross-reactivity with other triazine herbicides. Bushway's team also used EIA to determine the extent of atrazine, alachlor, and carbofuran contamination of well water in Maine.[328] The samples from 58 wells were analyzed by EIA and a SPE/HPLC reference method. Nine well waters that had previously tested negative for the three pesticides were spiked at 0.5, 1, and 5–10 ng/ml and used as positive controls. The EIA and HPLC results for the atrazine positive subsets from three surveys were in good agreement for the atrazine analyses ($R = 0.97$). Similarly, correlation coefficients of 0.94 and 0.96 were obtained for the carbofuran and alachlor methods, respectively. A fairly high incidence of false-positive results for the alachlor EIA was attributed to the possible presence of alachlor metabolites. The authors were unable to test their hypothesis because of the lack of reference metabolites. The alachlor EIA should still be valuable as a screening test since it would have facilitated the elimination of many alachlor free samples from further analysis. IMS's atrazine kit also has been validated using food samples and milk samples.[318, 329] The EIA for deethyl- and deisopropylatrazine had an inter-assay CV of 4.5% when used to analyze deethylatrazine controls on 10 different days.[185] EIA analysis of spiked tap water and environmental water was accurate, which indicated the absence of serious matrix effects. When Schneider et al. monitored atrazine and deethylatrazine levels in soil samples, the EIAs gave results that were comparable, although somewhat higher than those of an independent GC method.[186]

A total of 13 laboratories participated in an interlaboratory validation of EIAs for the detection of S-triazines in water samples.[324] Spiked and unspiked samples of ground water, drinking water, and surface water were analyzed; a reference laboratory used GC/MS. A variety of EIA kits were evaluated in the study including kits from IMS (Res-i-Mune and Res-i-Quant), Hock's laboratory, and Envirogard. Only a few of the laboratories were able to precisely determine the atrazine content of the samples. The main problems appeared be the inexperience of some of the participants and cross-reactivity by other triazines. In a later more rigorous

evaluation of the three best assay systems (Res-i-Quant, Hock's, and Envirogard), each of the IAs correctly identified the negative samples. Test kits that employed broad specificity ABs (Res-i-Quant and Envirogard) yielded higher than normal atrazine levels. The study's organizers made several useful recommendations: (1) IA laboratories should be certified and personnel must be highly trained; (2) an exact description of the assay's protocol should be available; (3) an assay's working range should be close to the middle of the calibration curve; (4) at least six calibration points should be used; (5) if the matrix is unknown, a fortification experiment should be undertaken; and (6) four parallel measurements should be made per sample to guard against erratic results.

Goh et al. reported that the IMS tube-based EIA can give variable results with standard solutions.[316] ANOVA indicated that most of the variability could be attributed to the within-assay replicates and probably resulted from technique problems or a lack of kit uniformity. Those researchers analyzed a set of 48 soil samples using GC and EIA; the results indicated good correlation ($R^2 = 0.97$) between the two methods despite a significant bias on the part of the EIA data that was mostly caused by dilution artifacts.[316] Thurman et al. compared the ability of the IMS tube assay and GC/MS technique to recover a group of triazine herbicides from surface water and ground water.[319] Both methods were comparable ($R \geq 0.91$) over the range 0.2 to 3 ppb. The correlation was maintained when the samples were enriched by SPE; the data were corrected for cross-reactivity factors. No false negatives were reported at a DL of 0.2 ppb. The IDAB-EIA for triazine that was developed by Hammock's team at UCLA was used to determine simazine in soil samples that were from an investigation of leaching from agricultural fields.[330] There was good correlation between the data produced by the EIA and an independent GC method ($R^2 = 0.82$ to 0.89), although there was evidence of some bias in the results. At levels below 100 ppb, the EIA gave higher values than the GC method; whereas this situation was reversed above 100 ppb. The performance of the same assay also has been validated by its originators; acceptable recoveries were obtained from spiked waster samples and from spiked soil extracts. The assay could tolerate up to 10% methanol in the extracts and no interference effects were observed.[186]

Sharp et al.[179] assessed the performance of their EIA for triazines by comparing the ability of the IA and a conventional GLC/nitrogen phosphorus detector (NPD) method to recover a range of atrazine spikes from water and soil samples. A paired *t*-test indicated there was no significant difference between the methods, although the EIA values for the soil samples were about 15% higher than the GLC values. Huber and Hock found no difference in the performance of the EIA for terbutryn when it was used to analyze filtered river water and tap water.[181] The EIA of Wust et al.[184] for atrazine corresponded well with a GC confirmation technique when used to analyze a set of water samples. Calibration curves that were prepared in various unfiltered and unbuffered environmental waters varied little from buffer controls when analyzed by the EIA of Harrison et al.[199] for atrazine. Shell Research's EIA for cyanazine was validated using a variety of atrazine- and cyanazine-contaminated samples.[137] The IA results agreed with those of a reference GC method, and the method has subsequently been used in monitoring applications.

The EIA for molinate was validated by the preparation of calibration curves in creek and rice field water.[229] Although shifted to the right, the curves prepared in matrix were parallel to the reference curve. The assay's precision was acceptable (intra-, 4%; inter-, <10%). The assay's performance was later verified by comparison with a GC method.[77,320] The IA and GC methods gave comparable recoveries of molinate (1 ppb to 1 ppm) from extracted tap, creek, ditch, and rice field water samples ($R^2 = 0.995$). Calibration curves that were prepared in soil extract were interference free when less than "5% soil extract" was used, although the data for low-level spikes showed elevated variability and a raised DL. The EIA and GC methods also compared well when used to analyze soil extracts and air samples from rice fields ($R^2 = 0.986$), although the EIA estimates were biased on the high side (up to 3×).

The practical usefulness of the molinate EIA was rigorously tested when the assay was used to study the herbicide's dissipation and distribution in a treated rice field.[331] Negative and positive controls were used to assess the EIA's reliability. The

water samples from the rice field were split and analyzed by GC and EIA. The assay calibration curve was repeatable (n = 56) and precise. The positive and negative controls did not exceed the mean ±2SD for the study's duration. ANOVA showed that the largest source of the positive control's variance was the replicate wells. The EIA's estimate of the amount of molinate that was added to the fortified samples was significantly different from the actual amount added. The GLC estimates did not exhibit this effect. Nevertheless, the GLC and EIA results for the field water samples were well correlated (R = 0.9).

Although more rugged than its heterologous counterpart, the homologous version of the EIA for maleic hydrazide was affected by residual matrix components when it was used to screen potato samples. The matrix effects depended on potato variety, the antibody, and the method of sample preparation.[224] Spike recoveries were more variable from the sample matrix than from blanks. Good correlation between the EIA and a colorimetric method (R = 0.92) was observed.

Fleeker validated two EIAs for 2,4-D using ground water samples that had been fortified with [^{14}C] 2,4-D.[171] The EIA data compared well with the scintillation control method (R = 0.99). Knopp et al.[172] used fortified human serum (5 to 250 ppb) to evaluate an RIA for 2,4-D; analyte recoveries were in the 94 to 104% range and the inter-assay CV% was a low 5.3. The RIA of Hall et al.[174] for 2,4-D was validated using fortified river water and urine (250 and 2500 ppb). The analyte recoveries were excellent and the intra-assay CV was less than or equal to 9%. Similar results were obtained for the same group's indirect EIA for the detection of 2,4-D. An EIA for clomazone yielded similar dose-response curves for spiked extracts of two soil types.[215] A correlation coefficient of 0.98 was obtained when the EIA of Dargar et al.[216] for clomazone was compared with a GLC method. The clomazone EIA was found to agree with the results of leaf damage bioassay tests when laboratory test soils were analyzed, although agreement was not as good for field soils. The inter- and intra-precision (CV) for a 10-ppb control were 16.7 and 12.8%, respectively. EIA efficiently recovered imazamethabenz spikes (12.5 to 200 ppb) from barley and wheat over the entire spiking range.[232] A mean recovery of 89.8% was obtained for four barley samples that were spiked with 12.5 ppb.

A set of 208 samples from rivers and water treatment plants, some spiked as positive controls, were used to assess the EIA for alachlor.[217] There was good agreement between the EIA and the confirming GC/MS technique (R = 0.84) (Figure 18), although the EIA was less accurate and precise (CV% = 10 to 40). The EIA data were reinterpreted using threshold levels of 0.5, 1.0, and 5.0 ppb. At the 1.0-ppb level, 99% of the negative samples were confirmed by GC/MS. Only 48% of the samples that were positive at the 1.0-ppb threshold were confirmed by GC/MS. Overall, the EIA could reduce the number of samples requiring confirmation by 71%. This is a good example of the ability of an IA to correctly identify negative samples in a screening application. The precision and accuracy of Ohmicron's magnetic particle-based EIA for alochlor were studied and found to be satisfactory by the replicate analysis of spiked water samples over a range of concentrations.[219] When a set of 272 ground water samples was spiked with 1 ppb alochlor, the mean recovery of all samples was 107%. The curves obtained by the dilution of positive samples were found to parallel the assay calibration curve, which indicates the absence of serious interferences. There was good agreement between the EIA and a GC/MS confirmation technique (R = 0.984). The EIAs for metolachlor were validated in a recovery study that used soils that were fortified with 20 and 50 ppb of analyte.[221] The mean recoveries for the direct and indirect assays were 98 and 89% respectively; the inter-assay CVs were 14.5 and 14%, respectively, over 40 assays. Some matrix interferences were noticed in the direct EIA. The EIA for bentazon was validated by spiking tap water with the herbicide (1 to 100 ppb) and then analyzing the samples by EIA and GC. The samples were preconcentrated by SPE using C8 cartridges. Analyte recoveries ranged from 99 to 118% (SD = 1.8 to 13.4) and there was excellent agreement between the two methods (R^2 = 0.95).[234]

The various IAs for paraquat have been validated using spiked serum (R = 0.95),[203] spiked soils (R = 0.97),[206,311] serum (R = 0.997 for the FIA),[203,213] plasma (R = 0.998),[201,332] and milk, potato, and ground beef.[212] Good agreement was established in a comparison of an EIA and RIA for paraquat (R = 0.96; n = 41).[204] The precision of the FIA for paraquat was established by the replicate analysis of low, medium, and high paraquat spikes in serum samples; the intra-assay precision was 6 to 9% and the inter-

assay precision ranged from 6.9 to 11.4%. This is excellent precision for a rapid screening assay.[213] Van Emon et al.[211,212] demonstrated parallelism between paraquat calibration curves that were prepared in the presence of different levels biological matrices. Paraquat recoveries from agricultural workers were generally higher for EIA than GC; moreover, the EIA afforded lower DLs.[211]

C. Insecticides

When used to analyze fortified soil samples, the chlordane EIA's precision was adequate for a screening assay (CV% <20) across the assay's analytical range (3.8 to 897 ppm).[237] The EIA was able to correctly detect the presence or absence of analyte in seven soil samples. Analysis of DDA in sets of spiked and nonspiked urine samples indicated close agreement between an EIA for DDA and reference GC and colorimetric methods.[267] Extracts of milk that had been fortified with diflubenzuron and BAY SIR 8514 and then analyzed by EIA yielded calibration curves that were parallel to and statistically indistinguishable from control curves.[248] The permethrin EIA was validated by demonstration of an approximately parallel relationship between the control curve and a curve that was prepared in meat extract.[240] There was good correlation between the observed and expected levels of permethrin for analyte levels greater than 50 ppb. The EIA for aldicarb was shown to be applicable to a variety of matrices such as stream water, plasma, urine, and citrus fruit juices; the dose responses were linear for the fortified matrices, although some low-level matrix interferences are apparent from the published curves.[268] The precision and accuracy of the magnetic particle-based EIA for aldicarb were established in a series of experiments that used spiked water from various sources.[220] An additive dose response was demonstrated when 160 water samples from across the U.S. were spiked with 30 ppb of aldicarb. A correlation coefficient of 0.93 was obtained when 14 ground water samples were analyzed by EIA and HPLC and GC confirmation methods.

When used to analyze spiked blood, an RIA for paraoxon showed good precision (9.5 to 12%) over the range 0.2 to 3.2 ng.[333] The ability of the EIA to determine 4-nitrophenol in SFE extracts of soil samples was verified by comparison with a GC method (R^2 = 0.999).[314] The ability of the same assay to detect parathion, after conversion to 4-nitrophenol, was similarly validated (R^2 = 0.99). In the latter regression analysis there was clustering of the samples at high and low parathion levels. The EIA for methoprene agreed well with an HPLC confirmation method when used to analyze wheat, flour, germ, bran, and tobacco extracts.[270, 271] The inter- and intra-assay precision of the EIAs for FN, CPM, and PIRM were studied and found to be satisfactory.[266] The intra-assay CV% was less than 15 for low, medium, and high pesticide wheat samples in the FN and PIRM assays, and less than 25 in the CPM assay. Subsequent modifications to the CPM assay improved its precision. The EIA methods and an independent GC method compared well when used to analyze the insecticide levels in whole wheat and milling fractions. The ELISA for *B. thuringiensis israelensis* endotoxin correlated well with a bioassay control method when used to analyze seven commercial formulations of the insecticide.[273]

D. Fungicides

The FIA's estimates of 2-ABZI in spiked water samples were within 5% accuracy in a small-scale experiment.[123] EIA and GC or LC-based methods were used to recover the following fungicides from a variety of foods and agricultural produce: MBC and benomyl,[277,279,334] metalaxyl,[280] triadimefon,[281] and iprodione.[282] In most cases, there was close agreement between the IA and the control method. The performance of an EIA for benomyl and thiabendazole was validated by assessing analyte recoveries from a variety of fortified commodities.[278] A GLC method and EIA gave comparable estimates of the fungicide fenpropimorph in spiked tap water and soil percolation water.[283] The EIA's estimates were higher for samples from a treated field, probably because the IA detects fenpropimorph and fenpropimorph acid, whereas only the latter is detected by GLC. The EIA for tetraconazole had excellent precision across its working range (<15%).[286]

VII. FUTURE PROSPECTS

Prospects for the future use of IAs in environmental laboratories are intimately linked to the

selection of suitable target analytes, the demonstrated application of several showcase IA methods, and the innovative coupling of the AB-AG interaction to emerging technologies from other disciplines.

A. Selection of Target Analytes

IAs can be developed for a wide variety of agrochemicals and industrial contaminants. The criteria used to select additional target molecules will have a key influence on whether or not environmental IAs are eventually accepted as routine tools. Proposed target molecules should be *difficult to analyze by conventional means*.[335] Since IAs do not depend on volatility, thermal stability, or the presence of chromophores,[85] they are well suited to compounds that are difficult to analyze by chromatographic methods.[4,11,22,85] Many analytes that usually require derivatization for detection by GC or LC methods can be readily detected by IA. There is a trend for the newer pesticides, especially biological agents, to be larger, more polar, and less volatile than their predecessors — which should make them suitable for analysis by IA.[11,39] IAs are typically most sensitive toward large molecules that have several polar groups.[138] Although ABs can be raised against haptens that are as small as 150 Da,[22] assay quality tends to be poorer for small molecules owing to the preponderance of low-affinity and low-specificity ABs.[129]

Ideally, the target molecule should be at least *moderately soluble in water*, both for ease of separation from lipid matrices and to facilitate binding with the ABs in the assay buffer. Hydrophilic molecules can be difficult to analyze by conventional methods.

There should be a *genuine need* for data on the target molecule's distribution and fate. Regulatory agencies, analytical chemists, and IA specialists could assist each other in the selection of target molecules by compiling a list of analytes for which screening techniques would be beneficial. Return on the initial investment and cost savings will be greatest for analytes that must be determined in many samples.[4,130] A lone technician can readily analyze more than 100 samples a day with many environmental IAs. IA efficiency can be further improved by the automation of individual assay steps using liquid handling work stations, automated micro-titer plate washers, and semiautomated plate readers or radiation counters.[16,31,138,336] Completely automated IA systems are now available for clinical applications,[337-339] some of which can process over 100 samples per hour.[338,339] Since IAs can be used to screen large sample sets at realistic costs,[17,39,77,130,132] they should facilitate large-scale surveys and monitoring programs that are currently unrealistic. Analyses that require the detection of multiple analytes in a few samples, however, are best undertaken by conventional means.

IAs are intended to complement rather than replace conventional analytical techniques. In screening applications, all positive samples and, if necessary, a statistical selection of negative samples should be confirmed by an independent technique (Figure 7).[340] Positive samples can be ranked in order of suspected residue levels, for prioritized confirmation. The confirmation of all positive samples may be unnecessary when the presence of a known contaminant is being monitored in an ecosystem with which the analyst is familiar, and which is known to contain no interferences; in which case, the IA would serve as a quantitative method. IAs are particularly useful for the identification of samples that exceed a threshold value and should be useful in the identification of waters that exceed contaminant guidelines. Particular care must be paid to samples that lie close to the cut-off point or that yield dubious results. Such samples should be reanalyzed by the IA (Figure 7) and submitted for confirmation analysis if doubts persist. Positive control samples can help maintain confidence close to the cut-off concentration.

B. Factors Affecting Implementation

IAs have yet to make a significant impact in routine environmental laboratories. In a submission to the Congress of the U.S., Office of Technology Assessment, Mumma and Hunter considered the constraints and opportunities of IAs in light of regulatory and legislative influences.[11] Regulatory agencies were criticized for being slow to encourage modern methods such as IAs. The agrochemical industry, it was argued, should be encouraged to share their IA data with the scientific community so

that more informed decisions can be made on the general usefulness of IA technology.

Approval of an IA by the relevant environmental regulatory agencies is a crucial prerequisite for its widespread use in routine laboratories. The majority of the assays reviewed in the present report must still be considered research tools rather than routine methods. Although the California Department of Agriculture, which has in the past supported the development and testing of IAs,[11,331] in a risk-taking move has initiated a program to monitor rice paddy water for molinate and thiobencarb by ELISA.[77] The FSIS in the U.S. is reported to have decided to incorporate IAs into its regulatory program. FSIS has selected a group of pyrethrin insecticides as its initial target compounds. Several other regulatory agencies in the U.S. have contracted out the development of new or improved IAs or have entered into agreement with university laboratories for the same purpose (for a list, see Reference 138). The use of some of the more promising IAs in routine applications and the rational development and distribution of ABs for additional target analytes would help to popularize environmental IAs.

Without doubt, the poor availability of ABs has retarded the acceptance of environmental IAs by routine analysts. Although ABs have been developed for numerous key analytes in recent years, those ABs are often unavailable to the scientific community. Inexperienced laboratories are naturally reluctant to undertake the time-consuming and somewhat risky development of ABs, particularly if scepticism about the applicability and general utility of environmental IAs persists.

Several manufacturers of agrochemicals have taken the lead in the production of ABs to their products; many of those sera are mentioned in the present review. Currently, about 50% of pesticide manufacturers are reported to have healthy IA projects.[2] Other manufacturers and producers of industrial contaminants would do well to follow this example. Once developed, sera could be made available to interested researchers through antibody banks,[78] as collaborative research gifts, or as commercially marketed reagents; sera that are not available for general evaluation and use are of little value. On this point, it is difficult to justify the attitude of agrochemical corporations that decline to make small quantities of sera to their pesticides available to government laboratories for research and evaluation purposes. In Europe, the possibility of supplying ABs and immunoaffinity columns through a centralized bureau is being studied.[341] Once ABs become more readily available, individual users will be free to develop and optimize their own assays. Alternatively, sera can be produced and marketed by the private sector. Profitability dictates that private corporations will, at least initially, develop assays for high-demand applications.[129]

Several companies now market IA kits for the detection of contaminants in food and in environmental matrices.[2, 130] ImmunoSystems Inc. of Scarborough, ME, was one of the first companies to offer IA kits for environmental contaminants. This energetically managed company has developed kits in tube (Res-I-Mune) and plate (Res-I-Quant) formats for a wide range of compounds including triazines, alachlor, aldicarb, benomyl, 2,4-D, and carbofuran. More than any other company, IMS has pioneered the development and use of commercial IA kits for environmental applications. Millipore, who initially distributed some of the IMS assay systems as their Enviro Gard line of screening tests, has now acquired IMS. The Millipore/IMS kits are available for the following analytes: triazines, cyclodienes, carbofuran, 2,4-D, aldicarb, alachlor, benomyl, picloram, PCP, methoprene, metalaxyl, captan, and PCBs. Several of the kits are available in tube and plate formats. Bio-Nebraska markets a kit version of their IA for the detection of mercury.[342] The kit can be used to test samples either in the field or laboratory. OHMICRON has developed a series of Rapid Pesticide Immuno Detection assays that use ABs immobilized on magnetic particles to effect phase separation. The assay format eliminates tube to tubes variation due to uneven AB coating.[343] Assays are available for triazines, aldicarb, alachlor, benomyl, 2,4-D, captan, carbofuran, cyanazine, metolachlor, carbaryl, and procymidone. Granite Division of Environmental Diagnostics Inc. sells rapid format IA kits for paraquat, but reports minimal sales thus far.[344] Westinghouse BioAnalytic, after having developed and distributed EIAs for PCP, triazines, carbofuran, aldicarb, and parathion, has now withdrawn from the marketplace. EnSys Inc. of North Carolina, how-

ever, is reported to have developed kits for the detection of PCBs, PCP, and total petroleum hydrocarbons.[345]

Commercial IA kits should offer a clear-cut cost advantage over conventional analytical methods, even after allowing for replicate analyses and the inclusion of control samples. Exorbitant pricing policies will at best force laboratories with extensive analytical requirements to develop in-house kits, or, at worst, to ignore IA technology altogether. On the other hand, competitive pricing will make IA kits more attractive to laboratory managers, facilitate the replicate analysis of samples, help control overall costs, and improve data comparability. Although the market for environmental IAs has suffered its share of growth pains, once the first few assays are firmly established additional users should emerge to solidify sales and pave the way for future growth.

C. Future Growth Areas

The low cost of IA technology should be particularly attractive to Third World Countries for whom analytical costs are virtually prohibitive. Since IAs can often be used to analyze body fluids with little or no clean-up, they could be used to screen groups of humans following accidental or occupational exposure to harmful organic contaminants.[39,85,164,268] EIA has been successfully used to monitor agricultural workers for exposure to paraquat in a pilot study.[211] In Germany, the RIA for 2,4-D was used to monitor residue levels in the urine of exposed workers from the agricultural and forestry sectors.[346] EIA also has been used to measure dermal and respiratory exposure in operators of all terrain vehicle towed sprayers.[347] IAs are also ideally suited for the detection of biomarkers of exposure to environmental contaminants (for a review, see Reference 348). Several assays that have been developed for the detection of BP-DNA adducts have been used to screen samples of people that are considered to have high exposure to that contaminant. A novel application of IA technology has been the use of EIA to measure increases in cytochrome P4501A1 levels in cod larvae and juveniles that were exposed to crude oil. The method may prove useful not only in detecting biological effects in exposed natural populations, but also as an accessory to various lethal and sublethal toxicity tests.[349] Several EIA formats, particularly the coated tube assays, are ideal for field applications such as monitoring adjacent waters during pesticide applications and the selection of meaningful sampling sites during surveillance studies.

VIII. RELATED TECHNOLOGIES

A. ImmunoAffinity Chromatography (IAC)

The selective AB-AG reaction can be used to extract hapten molecules from complex solutions. ABs have been used in a novel extraction system to actively extract paraquat from macerated glass fiber filters.[210,211] In IAC, the ABs are attached to a rigid or semi-rigid support which is then packed in a small column. Aqueous phase sample is passed through the column bed, the immobilized ABs selectively remove the target molecules from solution, and the analyte is subsequently desorbed and eluted. A variety of preactivated support materials are commercially available. The activated columns are stable and can usually be reused many times.[350] For high pressure IAC (HPIAC) techniques, the ABs are immobilized on rigid supports that can withstand up to 1000 psi.[351] IAC combined with other clean-up methods such as gel permeation chromatography (GPC) could be used to selectively remove contaminant residues from environmental matrices. IAC has been used to extract the steroid oestradiol-17b from plasma and milk.[352] Hamers and Paulussen developed an automated column switching system to link an IAC column with a C18 HPLC column for the isolation of nortestosterone from biological samples.[353] Several hundred samples could be analyzed on each IAC column. Immunocolumns have also been coupled to on-line to liquid chromatography columns for the isolation and determination of aflatoxins[354] and estrogen analytes,[355] and via a reversed phase precolumn, to an on-line capillary GC system for the determination of nortestosterone in large-volume urine samples.[356] Researchers at Monsanto Agricultural Company used IAC for the isolation of cytokinins.[357] The column's capacity was increased by the use of pu-

rified IgG, and the method had a dynamic capture efficiency of 100%. Co-extracted molecules were not a problem when a MAB-based IAC system was used to enrich chloramphenicol from swine muscle tissue with 100% recoveries.[358] IAC has been used to isolate many other analytes such as aflatoxin Mi from milk,[359] chloramphenicol from milk (1 l) and eggs,[360] and nortestosterone from defatted meat samples.[361] The availability of continuous supplies of MABs could help make IAC an attractive option for analytical chemists. The potential benefits of IAC warrants a rigorous evaluation of the technique using some trial environmental analytes for which ABs have already been developed.

B. Immunosensor Probes and Flow Injection Immunoanalysis (FIIA)

There is much current interest among clinical chemists in the development of immunosensor probes for the *in vivo* detection of analytes.[362,363] Similar probes could be used in nonclinical applications, such as the monitoring of effluents or receiving waters.[362] Bright et al.[364] described an evanescent wave immunosensor that consisted of AB fragments that were labeled with fluorescein and then immobilized on the end of a fiber optic probe. When analyte bound to the ABs, a three- to fivefold increase in laser excited fluorescence occurred because of shielding of the fluorescein from its solvent. The sensor could be regenerated more than 50 times and was evaluated using HSA as analyte. It is also possible to immobilize the hapten on the probe surface and to monitor the binding reaction using ABs that are labeled with a fluorescent marker.[365,366] Such a system has recently been reported for the detection of parathion.[367] A casein-parathion conjugate was immobilized on the probe surface, and the binding of anti-parathion ABs to the hapten was monitored using a fluorescein isothiocyanate-labeled second AB. The probe was calibrated using a range of parathion standards. The probe could detect as little as 0.3 ppb of parathion, which is excellent sensitivity. Paraoxon was detected at 100 times higher concentrations. The probe discriminated against other anti-cholinesterase insecticides.

In an alternative format, unlabeled primary ABs are immobilized on the probe; after contact with the analyte, the probe is exposed to a fluorescein-labeled second AB.[368,369] The analyte level is proportional to the intensity of the fluorescent signal that can be induced by an excitation laser. This sandwich format is unsuitable for small haptens and would have to be modified for use with most environmental contaminants of current concern.

Biotronics Systems Corporation has developed a capacitive affinity sensor for the detection of PCP; the probe is intended for use in the continuous monitoring of effluent or receiving water.[370] The probe consists of a sensor cell that contains capture ABs which are retained by a size-selective membrane. Hapten molecules are covalently bound to the sensor's surface. When the probe is in contact with sample, free analyte diffuses through the membrane and displaces some of the ABs, which are bound to the immobilized hapten. A change in the dielectric constant between two capacitor plates results, which generates a measurable change in the cell's capacitance.

A flow immunosensor has been reported that could rapidly detect as little as 29 pmol of 2,4-dinitrophenol-lysine/200 µl in continuous flow conditions;[371] this probe should be readily adaptable for use with other analytes in water samples. In research that opens the way for the development of multichannel immunosensors, Hlady et al.[372] demonstrated the spatially resolved detection of AB-AG reactions at a solid/liquid interface by means of excited antigen fluorescence. There are still many formidable problems associated with the development of immunosensor probes,[390] such as denaturation of the ABs, nonspecific binding, irreversible binding of competing compounds,[389] and interference from impurities in the sample that fluoresce at the same wavelengths as the tracer;[365,] these problems must be solved if immunoprobes are to be used outside the research environment.[373]

The proven utility of electrochemical techniques, such as flow injection analysis combined with electrochemical detection, has sparked interest in the development of electrochemical IAs (reviewed in References 74 and 374). Two types of labels have been explored: (1) enzyme labels that catalyze the production of electroactive compounds and (2) labels that are themselves electroactive. Several promising assays for clinically important analytes such as digoxin (DL: 50 pg/ml) have been developed. Elec-

trochemical IAs are free from many matrix interference problems and afford DLs in the low picogram per milliliter range with samples that are as small as 20 µl. Some recent research on this topic has led to an electrochemical EIA that can be used for the rapid and sensitive detection of toxic substances in solution.[375] The traditional model compound 2,4-DNP-L-lysine was used as the analyte for the initial research. Basically, the system is a homogeneous enzyme-linked electroimmunoassay. Anti-analyte ABs are allowed to bind to an antigen mediator complex. Introduction of analyte displaces the AG-mediator in a competitive manner. The displaced mediator assists the transfer of electrons from a gold electrode to the HRP. Cycling of the mediator between the electrode and the enzyme generates an electrical current that can be measured. The immunosensor electrode had a linear response from 0 to 1 µ*M*. Similar sensor systems should be feasible for many small molecular weight environmental contaminants, although, once again, there are many practical problems that have yet to be overcome.[376]

The adaptation of flow injection analytical techniques for use with biochemical reagents such as ABs and enzymes have depended to date on adequate contact time between the reagent and analyte molecules which is achieved by the use of stopped-flow techniques.[377] Two recently published reviews have surveyed the FIIA methods proposed thus far.[377,380] The first FIIA method for the detection of a pesticide in water used an anti-atrazine serum which was immobilized on immunoaffinity membranes (Figure 19).[379] The membrane is automati-

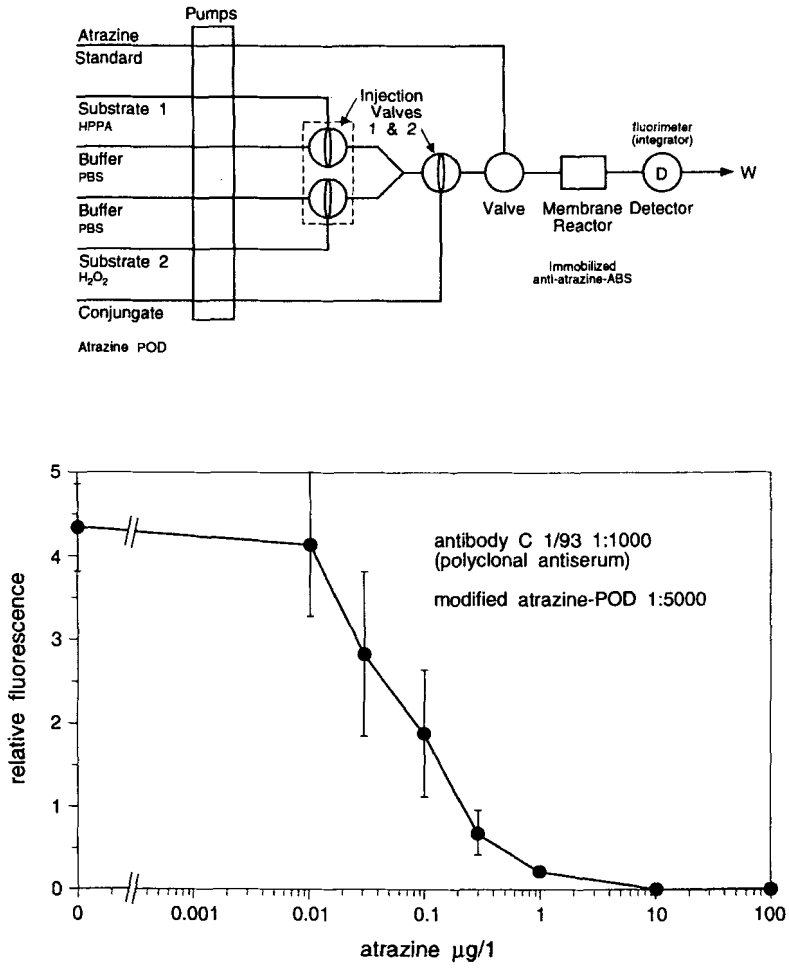


FIGURE 19. Kramer and Schmidt's FIIA system for the determination of atrazine. POD, horse radish peroxidase; HPPA, hydroxyphenyl propionic acid. (Taken from *Biosensors & Bioelectronics,* Elsevier Advanced Technology: Oxford, England. With permission.)

cally changed after each assay. A "stop-go" sequence was used to pump the reagents through the membrane reactor chamber and a fluorometer was used to detect the products of a peroxidase tracer. The calibration curve was linear from 0.02 to 0.3 ng/ml and the method had a DL of 0.02 ng/ml. The FIIA's precision was lower than that of the corresponding EIA, which may have several causes: the nature of the FIIA reaction, the use of nonequilibrium conditions, and the immobilization technique. Another promising FIIA system uses ABs that are immobilized on magnetic beads as the main reagent in the reactor.[380] An electromagnetic field is used to keep the beads stationary while the other reagents flow through the reactor coil. The reagents are sequentially injected in a competitive heterogeneous sequence, and the amount of unbound label is used to deduce the analyte concentration.

C. Multianalyte IAs

Theoretical and practical considerations restrict the use of current IAs to single or dual analytes.[381] Multianalyte IAs would find ready application in clinical and environmental laboratories.[61,382,383] It appears that assay formats that are based on labeled ABs offer the best possibility for the detection of multiple analytes in small-volume samples. Ekins and co-workers,[61,382,384] in a recent series of publications, have described the principles underlying a multianalyte microspot IA technique (Figures 20 and 21). The authors estimate that the technique, which is in its infancy, has the potential to detect as many as 10^6 different analytes in a 100-µl sample. The proposed technique would use fluorescent labels, which are about the most active tracers available. The ABs are to be located in spatially separated microspots that collectively form an array on the surface of a microtiter well or of a probe. In practice, an array size appropriate to the task at hand would be used. Each microspot could be directed against a different analyte. When a small number of "capture" ABs are exposed to a small-volume sample, the fraction of AB binding sites that become occupied is directly proportional to analyte concentration and is independent of the quantity of capture ABs.[382] A labeled second AB can be used to detect either bound AG or unoccupied ABs (Figures 20 and 21).[61] Both sets of ABs can be labeled with rare earth fluorophores[81] that have distinct emission patterns;[44] the antibody occupancy can be expressed as a ratio of the two signals (Figure 21). The fluorophores would be quantified using time-resolved fluorometric detectors.[31] The practical application of the technique awaits the development of automated instruments that use spatially accurate excitation lasers and photon detectors. A theoretical analysis indicated that the noncompetitive microspot array assays should have a sensitivity of 4×10^{-15} M/l — which betters conventional RIA-like assays, which have a theoretical maximum sensitivity of about 10^{-14} M/l regardless of the tracer system.[44,47,61,382] The basic principles underlying multianalyte microspot IAs have been verified by the use of a laser scanning confocal microscope.[47,61] This novel assay format has the potential to revolutionize environmental IAs by facilitating the detection of multiple analytes in a single test, while improving assay sensitivity. The sensitivity of most competitive IAs usually falls short of what is theoretically achievable. At this time, it appears that the noncompetitive micro-spot IAs may offer the best prospect of multianalyte detection at previously unsurpassed levels.[82]

The first practical multianalyte IA was developed for the simultaneous assay of four clinically important analytes in serum.[385] Four polystyrene discs were coated with ABs and attached to either side of a carrier stick. Occupied AB binding sites were measured using a sandwich technique in which a second biotin-labeled AB bound to the AB immobilized analyte molecules. The biotin-labeled second AB was quantified by means of streptavidin labeled with a europium chelate. Kricka[383] has identified the following problems that will have to be solved before multianalyte IAs can become routine analytical tools: the possibility of cross-reactions, and difficulties in optimizing the assay ranges for individual analytes. The former problem can probably be best addressed by the use of high-specificity MABs. Presumably, multianalyte calibration standards could be used to calibrate each probe or assay. These are clear grounds for much additional research into the practicality and potential of multianalyte IAs for environmental contaminants.

IX. CONCLUSIONS

A decade of solid research into the use of IAs for the detection of environmental contaminants has

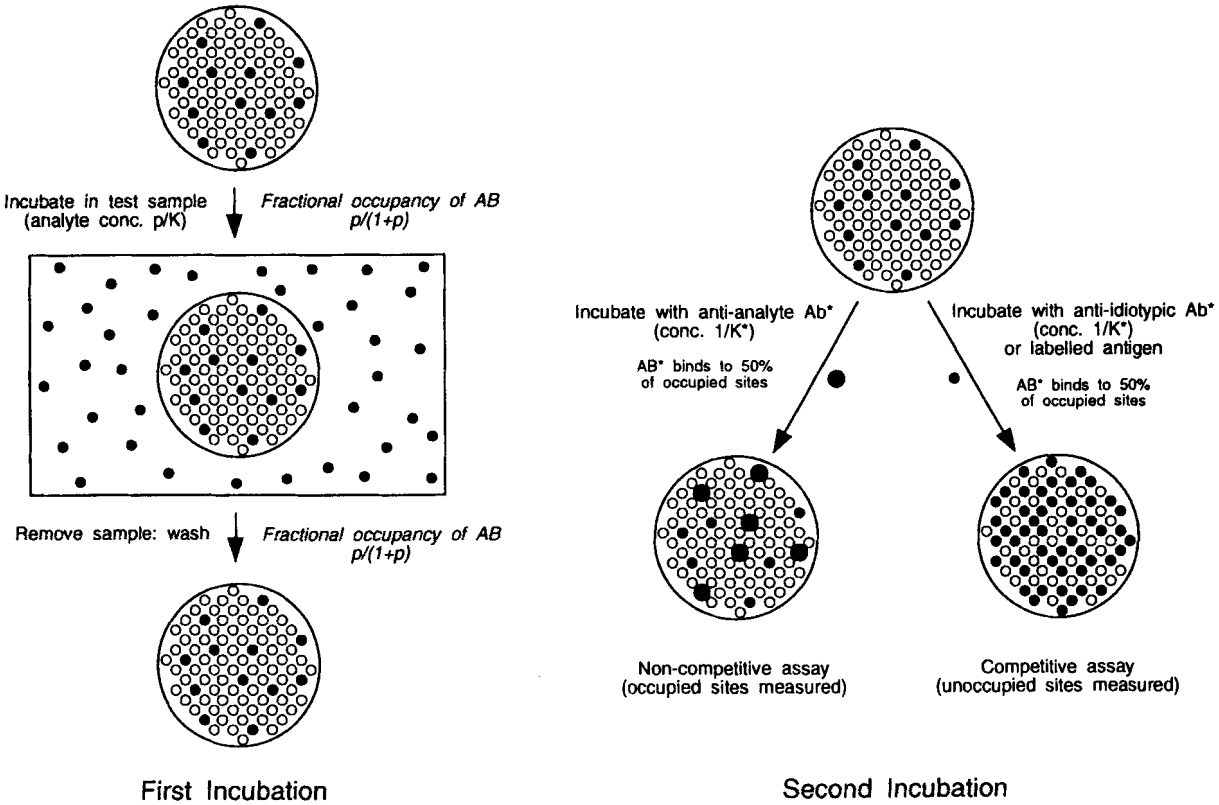


FIGURE 20. Microspot immunoassay. First incubation: the fractional occupancy of AB binding sites is proportional to the analyte concentration in the sample solution. Second incubation: the second AB reacts with occupied (noncompetitive) or unoccupied sites (competitive). (Reprinted with permission from Ekins et al., *Clin. Chim. Acta.* **1990,** *194,* 91–114; Elsevier Science Publishers.)

produced many IAs that can be used to detect low-level analytes in environmental matrices, often with little or no sample preparation. In several cases, the AB's specificity has been optimized so that detection of either a target compound or a group of contaminants is favored. Many of these assays are worthy of serious evaluation in routine settings because they combine sensitivity and precision, together with speed and low cost.

The development of environmental IAs has not been problem free, however. Although it has been possible to assay polar analytes in aqueous samples or in extracts of solid matrices with minimal cleanup, the assay of hydrophobic analytes in extracts of fatty samples has proven more difficult. The difficulty is caused by the tendency of residual lipids to shield the analyte from AB binding sites. The problem of residual interferences, combined with the lack of high activity tracer systems, has prevented IAs from meeting the ultra-trace analytical requirements for classes of compounds such as the HAHs. Research into the performance of ABs in anhydrous organic solvents and the development of high-activity fluorescent tracer systems may help to solve these problems. Innovative assay designs that are based on noncompetitive IA formats or that reduce the levels of AB and tracer in competitive IAs can also help to improve assay detection limits.

Among the factors that have impeded the widespread use of IAs in environmental laboratories are the paucity of full-scale and rigorous validation studies, the lack of certification and official approval, the high initial cost of assay development, and the previously poor availability of ABs to important pollutants. Active interest in IAs will increase as the foregoing difficulties are overcome — the converse applies.

Research into the development of new environmental IAs should focus on analytes that are either difficult and costly to analyze by conventional means,

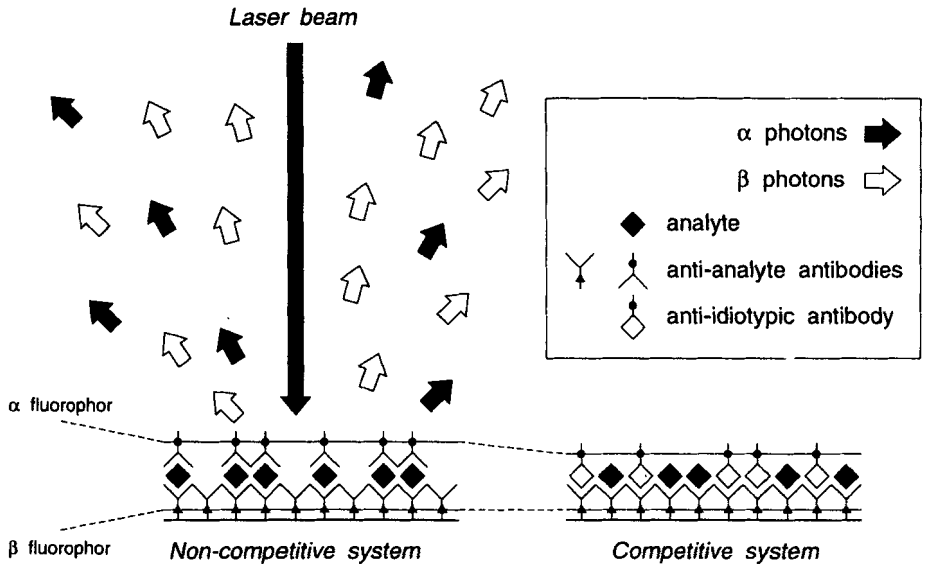


FIGURE 21. Basic principle of dual-label, ambient-analyte, immunoassay relying on fluorescent labeled ABs. The ratio of α and β fluorescent photons emitted reflects the fractional occupancy and depends solely on the analyte concentration to which the probe has been exposed. It is unaffected by the amount or distribution of antibody coated (as a monomolecular layer) on the probe surface. (Reprinted with permission from Ekins et al., *Clin. Chim. Acta.* **1990,** *194,* 91–114; Elsevier Science Publishers.)

or for which there is a strong analytical demand. Assay kits or reagent ABs for such analytes have the best chance of stimulating analysts to become involved with IA technology.

Effective distribution of ABs to high-demand analytes is essential if IAs are to become more widely used for routine analytical tasks. University and corporate laboratories can foster confidence and interest in environmental IAs by initially distributing sera on a collaborative basis. After a serum's potential has been established for a variety of matrices and applications, the ABs can be produced on a large scale and then widely distributed through AB banks or by the commercial distribution of IA kits or AB reagents.

Much effort is being made to lower the cost of conventional analytical methods through the use of innovative techniques, automated analyses, and miniaturization. Thus, it is doubtful that marginal cost savings will suffice to induce analysts to adopt an IA screening philosophy in the future. Unless the cost savings are attractive, the analyst will, not unreasonably, opt for a conventional method. Consequently, it is important that IA kits for environmental analytes be reasonably and competitively priced. On this point, a realistic cost analysis for an IA must make allowance for adequate replication and the inclusion of negative and positive control samples.

Present efforts by regulatory agencies and others to establish guidelines for the validation and approval of screening assays will most likely lead to the approval and subsequent widespread use of some of the more promising environmental IAs. This process should help accelerate efforts to have other IAs approved. Certification is crucial if IAs are to develop beyond the stage of being interesting research tools for use in pilot studies. The recently published validations of IAs for analytes such as the S-triazine herbicides should increase confidence in the ability of IAs to deal with real world samples.

The ability to detect multiple analytes in small-volume samples without the need for sample preparation is an exciting prospect. A couple of first-generation multianalyte IAs have already been announced for clinical analytes. Although much work remains, it is clear that the successful development of sensitive multianalyte IAs for clinical analytes will have immediate implications and spin-off benefits for environmental IAs.

There is a clear need for screening techniques that will help to lower the sample burden for environmental laboratories. The evidence suggests that, for the foreseeable future, this requirement shall persist and grow. Cost-effective screening technologies, such as IAs , that can improve analytical efficiency should be enthusiastically explored. The main users of environmental IAs will be analytical laboratories that routinely process large sample sets. Analysts who wish to explore what may be an unfamiliar technology can start by using commercial IA kits to screen sample sets. Until confidence is gained, samples can be processed using both conventional and IA techniques, after which only a random selection of negative samples and all positive samples need be confirmed. The list of analytes for which ABs and IA kits are available is already impressive and is certain to lengthen over the next 5 years.

EIAs and CIAs are readily incorporated into kits that are suited to field use by operatives with minimal skills; these kits provide mainly qualitative data. Quantitative IA kits are best used by technicians who have mastered the practice of systematic work, are aware of the underlying scientific principles on which the particular kit is based, and who can be relied upon to implement routine quality assurance practices. The use of portable IAs for the identification of contaminated waters, soils, or sediments, and for monitoring the application of agrochemicals shall probably increase in the coming years. Once a contaminated body of water is identified, additional samples can be collected and returned to the laboratory for screening by a more complex semiquantitative IA. Apart from reducing the numbers of negative samples that are collected, this strategy also can help to broaden the scale and scope of many environmental studies.

Interest in emerging immunotechnologies such as FIIA, electrochemical immunoassays, immunosensors, and IAC is also strong. The application of FIIA to environmental analytes offers the prospects of shorter analyses, ease of automation, and assay miniaturization. Although still in the early stages of development, immunosensors could become important tools because of their potential to continually or intermittently monitor effluents or receiving waters for the presence of low-level contaminants. When automated and interfaced with a computer, such an immunosensor would in essence be a robotic analyst. Multianalyte probes or multiple single-analyte probes could be attached to a single robot.

A spate of recent European publications on the use of IAC for the isolation of mycotoxins, antibiotics, anabolic steroids, and pharmaceuticals points to a fairly obvious use for some of the sera that have been generated for environmental contaminants. Immunoaffinity columns could provide a ready means of selectively isolating trace environmental contaminants from a variety of matrices — particularly aqueous samples. It can only be a matter of time before research on this promising topic receives serious support.

In conclusion, IAs are a useful analytical tool that can complement conventional analytical methods. Although the technique is not problem free, none of its identified shortcomings appear to be insurmountable. Interested analysts should take the initiative and explore IA's potential in environmental applications. The potential value of a selective and cost-effective screening technology far outweighs any associated risk. The overall prognosis is that environmental IAs may eventually become as valued by residue analysts as their counterparts are by clinical chemists.

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APPENDIX

Abbreviations used:

ABs: antibodies
2-ABZI: 2-aminobenzimidazole
AChE: acetylcholinesterase
ACN: acetonitrile
AG: antigen
ANOVA: analysis of variance
AOAC: Association of Official Analytical Chemists

BGG: bovine gamma globulin
BLS: Blasticidin S
B_0: reference tubes in IAs (zero analyte bound)
BP: benzo-a-pyrene
BSA: bovine serum albumin
CIA: chemiluminescence immunoassays
CR: cross-reactivity
CDI: carbodiimide
CMC: 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-4-toluene sulfonate
CONA: conalbumin
CPMe: chlorpyrifos-methyl
CV: coefficient of variation
CYP: dichlorovinyl cyclopropane carboxylic acid
2,4-D: 2,4-dichloro-phenoxyacetic acid
DAB: double antibody (technique)
DCC: dextran-coated charcoal
DCP: 0,0-diethyl-0-[4-carboxyethyl-phenyl] phosphate
DDA: 2,2-*bis*(*p*-chlorophenyl) acetic acid
DDT: 1,1,1-trichloro-di-(4-chlorophenyl)ethane
DiCC: dicyclohexylcarbodiimide
DL: detection limit
DMF: dimethylformamide
DMSO: dimethyl sulfoxide
DNT: 2,4-dinitrotoluene
DTP: 2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazoyl-1-yl)propanol
DTT: dithiothreitol
ECD: electron capture detector
EDC: 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide
EIA: enzyme immunoassay
ELISA: enzyme linked immunosorbent assays
EMIT: enzyme-mediated IA technique
FIA: fluoroimmunoassay
FIIA: flow injection immunoanalysis
FN: fenitrothion
FSIS: Food Safety and Inspection Service of the U.S. Department of Agriculture
GLC: gas liquid chromatography
GPC: gel permeation chromatography
HAH: halogenated aromatic hydrocarbon
HPIAC: high pressure IAC
HPLC: high performance liquid chromatography
HSA: horse serum albumin
I_{50}: concentration of analyte that causes 50% inhibition in an IA
IA: immunoassay
IAB: immobilized antibody
IAC: immunoaffinity chromatography
IAsph: antibodies immobilized on plastic spheres
ICI: Imperial Chemical Industries
IDAB: immobilized DAB assay format
IgG: immuno-gamma-globulin
IH: immobilized hapten
IRMA: immunoradiometric assays
IUPAC: International Union of Pure and Applied Chemistry
KLH: keyhole lymphocyte albumin
GC: gas chromatography
GLC: gas liquid chromatography
HPIAC: high pressure immunoaffinity chromatography
KLH: keyhole limpet haemocyanin
MABs: monoclonal antibodies
MATP: methyl phosphonic acid, *p*-aminophenyl 1,2,2-trimethyl-propyldiester
MB: methabenzthiazuron
MBC: methyl 2-benzimidazolecarbamate
MBS: N-(*m*-maleimidobenzoyloxy)succinimide
MIB: 2-methylisoborenol
MW: molecular weight
MS: mass spectrometry
na: not applicable
NHS: N-hydroxysuccinimide
NPD: nitrogen phosphorous detector
NSB: tracer that is nonspecifically bound
OD: optical density
OVA: ovalbumin
PA: protein-A
PABs: polyclonal antibodies
PAHs: polycyclic aromatic hydrocarbons
PBA: 3-phenoxybenzoic acid
PBS: phosphate buffered saline
PCBs: polychlorinated biphenyls
PCDDs: polychlorinated dibenzo-*p*-dioxins
PCDFs: polychlorinated dibenzofurans
PCP: pentachlorophenol
PEG: polyethylene glycol
PM: pirimiphos-methyl
PP: precision profile
PT: pinch test
PQL: practical quantitation limit
RSA: rabbit serum albumin
RIA: radioimmunoassay
SD: standard deviation of the mean
SFE: supercritical fluid extraction
SPE: solid phase extraction
2,4,5-T: 2,4,5-trichloro-phenoxyacetic acid

TFZ: thiabendazole
TYG: thyroglobulin
UCLA: University of California, Los Angeles

Glossary

Affinity: A measure of the strength of the binding reaction between an antigenic determinant and its antibody.

Antibodies: Protein molecules that are produced by vertebrate immune systems as a defense against foreign substances (antigens). Antibodies bind to specific sites on the foreign substance. The antibodies used in immunoassays belong to the IgG class of protein molecules.

Antiserum: Serum that contains antibodies to a target antigen.

Antigen: Any molecule that can bind specifically to an antibody's binding site.

Antigenic determinant: Area of the antigen that is selectively recognized by an antibody.

Avidity: A measure of the integrated strength of the binding reactions between antibodies and antigens. The avidity of a pure antibody refers to the combined strength of its interactions with antigenic determinants on the antigen. Avidity takes into account AB-AG affinity, multivalency of the AB or AG which can stabilize AB-AG complexes, the arrangement of the AB-AG complex, and differences between the determinants on an AG. In the case of an antiserum which contains polyclonal antibodies, avidity refers to the combined strength of the individual AB-AG reactions. The terms avidity and affinity have been rather loosely used in the literature.

B/B_0: The ratio of the amount of signal measured in the presence of analyte to the amount measured in the absence of analyte.

Binder: In the case of immunoassays, the antibodies are the binder molecules.

Carrier protein: A protein molecule to which a hapten is attached in order to render it immunogenic.

Coating antigen: Antigen that is coupled to carrier protein and is then immobilized on a solid phase. Used in many EIA formats.

Hapten: A small molecule that can elicit an immune response when conjugated to a carrier protein.

Immunogen: A substance that can elicit an immune response in a host species; induces antibody production. Generally a high molecular weight protein or carbohydrate.

Ligand: In the case of immunoassays, the free hapten, the analyte, the coating antigen, and the labeled hapten are often referred to as ligands.

Monoclonal: A highly pure antibody that is produced by a single lymphocyte clone. Each monoclonal antibody binds a single defined antigenic site on the immunogen. Since monoclonal antibodies can cross-react with other molecules, their specificity should be ascertained.

Plasma: Blood from which the corpuscules have been removed.

Polyclonal: Complex mixture of distinct antibodies each produced by individual B lymphocyte clones. The individual clonal antibodies may bind with a broad range of affinities to different sites on the immunogen.

Serum: The fluid obtained from clotted blood or plasma, i.e., blood minus the blood cells and fibrin.

REFERENCES

1. Sherma, J. *Anal. Chem.* **1989**, *61*, 153R.
2. Klausner, A., *Biotechnology*. **1987**, *5*, 551.
3. Yalow, R.S.; Berson, S.A. *Nature*. **1959**, *184*, 1648.
4. Hammock, B.D.; Mumma, R.O. *Recent Advances in Pesticide Analytical Methodology*; J. Harvey Jr., G. Zewig, Eds.; American Chemical Society Publications: Washington D.C., 1980; pp. 321.
5. Hunter, W.M. *Handbook of Immunology*; D.M. Weir, Ed.; Blackwell Sci. Publ.: Oxford, 1973; pp. 17.1.
6. McCormick, D.J.; Schmitz, H.E. *Molecular Immunology*; M.Z. Atassi, C.J. Van Oss, D.R. Absolom, Eds.; Marcel Dekker: New York, 1984; Chapter 19.
7. Midgely, A.R.; Niswender, G.D. *Acta Endocrin.* **1970**, *147*, 320.
8. Chapman, D.I. *Chem. Britain.* **1979**, *15*, 439.
9. Clifford, M.N. *Immunoassays in Food Analysis*; B.A. Morris, M.N. Clifford, Eds.; Elsevier: London, 1985; pp. 3.
10. Morgan, M.R.A. *Tetrahedron*. **1989**, *45*, 2237.
11. Mumma, R.O.; Hunter, K.W. Jr. *Pesticide Residues in Foods: Technologies for Detection*; Congress of the U.S.A., Office of Technology Assessment, 1988; pp. 171.
12. Tonegawa, S. *Sci. Am.* **1985**, *253*, 122.
13. Galfre, G.; Butcher, G.W. *Immunology in Plant Science*; T.L. Wang, Ed.; Cambridge University Press; Cambridge; 1986; pp. 1.

14. Harlow, E.; Lane, D. *Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, New York; 1988; pp. 726.
15. Coghlan, A. *New Scientist.* **1991**, *129*, 34.
16. Tijssen, P. *Practice and Theory of Enzyme Immunoassays*; Elsevier: Amsterdam, 1985; pp. 549.
17. Lankow, R.K.; Grothaus, G.D.; Miller, S.A. *Biotechnology in Agricultural Chemistry*; H.M. LeBaron, R.O. Mumma, R.C. Honeycutt, J.H. Duesing, J.P. Phillips, M.J. Haas, Eds.; ACS Symposium Series #334, ACS: Washington, D.C., 1987; pp. 228.
18. Van Oss, C.J.; Absolom, D.R. *Molecular Immunology*; M.Z. Atassi, C.J. van Oss, D.R. Absolom, Eds.; Marcel Dekker: New York, 1984; pp. 337.
19. Ekins, R. *Immunoassays for the 80s*; A. Voller, A. Bartlett, D. Bidwell, Eds.; University Park Press: Baltimore, 1981; pp. 5.
20. Skelley, D.S.; Brown, L.P.; Besch, P.K. *Clin. Chem.* **1973**, *19*, 146.
21. Corrie, J.E.T. *Immunoassays for Clinical Chemistry*; W.M. Hunter, J.E.T. Corrie, Eds.; Churchill Livingstone: Edinburgh, 1983; pp. 469.
22. Aherne, G.W. *Int. J. Environ. Anal. Chem.* **1985**, *21*, 79.
23. Landsteiner, K. *The Specificity of Serological Reactions*; Harvard University Press: Cambridge, MA, 1945; pp. 330.
24. Parker, C.W. *Radioimmunoassay of Biologically Active Compounds*: Prentice Hall: Englewood Cliffs, NJ, 1976; pp. 239.
25. Warren, J.T.; Gilbert, L.I. *Immunological Techniques in Insect Biology*: L.I. Gilbert, T.A. Miller, Eds.; Springer Verlag: New York, 1988; pp. 181.
26. Erlanger, B.F. *Methods in Enzymology (70) Immunochemical Techniques*: H. Van Vunakis, J.J. Langone, Eds. Academic Press: New York, 1980: pp. 85.
27. Weiler, E.W.; Eberle, J.; Mertens, R.; Atzorn,R.; Feyerabend, M.; Jourdan, P.S.; Arnscheidt, A.; Wieczorek, U. *Immunology in Plant Science*; T.L. Wang, Ed.; Cambridge University Press: Cambridge, 1986; pp. 27.
28. Wust, S.; Hock, B. *Anal. Lett.* **1992**, *25*, 1025.
29. Albro, P.W.; Luster, M.I.; Chae, K.; Chaudhary, S.K.; Clark, G.; Lawson, L.D.; Corbett, J.T.; McKinney, J.D. *Toxicol. Appl. Pharmacol.*, **1979**, *50*, 137.
30. Erlanger, B.F.; Beiser, S.M.; Borek, F.; Edel, F.; Lieberman, S. *Methods in Immunology and Immunochemistry*; C.A. Williams, M.W. Chase, Eds.; Academic Press: New York, 1967; pp. 1, 144.
31. Chard, T. *An Introduction to Radioimmunoassay and Related Techniques*; Elsevier Amsterdam, 1987; pp. 274.
32. Bauminger, S.; Wilchek, M. *Methods in Enzymology (70) Immunochemical Techniques*; Academic Press: H. Van Vunakis, J.J. Langone, Eds.; Academic Press: New York, 1980; pp. 151.
33. Nisonoff, A. *Methods in Immunology and Immunochemistry*; C.A. Williams, M.W. Chase, Eds.; Academic Press: New York, 1967; pp. 1, 120.
34. Roe, R.M. *Pesticides and the Future: Toxicological Studies of Risks and Benefits, Reviews in Pesticide Toxicology*; E. Hodgson, R.M. Roe, N. Motoyama, Eds.; North Carolina State University, Raleigh, NC, 1991; pp. 273.
35. Butt, W.R. *Practical Immunoassay*; W.R. Butt, Ed.; Marcel Dekker: New York, 1984; pp. 1.
36. Hum, B.A.; Chantler, S.M. *Methods in Enzymology (70) Immunochemical Techniques*; H. Van Vunakis, J.J. Langone, Eds.; Academic Press: New York, 1980; pp. 104.
37. Hermann, B.W. *Immunological Techniques in Insect Biology*; L.I. Gilbert, T.A. Miller, Eds.; Springer-Verlag: New York, 1988; pp. 135.
38. Morris, B.A. *Immunoassays in Food Analysis*; B.A. Morris, M.N. Clifford, Eds.; Elsevier: London, 1985; pp. 21.
39. Cheung, P.Y.K.; Gee, S.J.; Hammock, B.D. *The Impact of Chemistry on Biotechnology*; M. Phillips, S.P. Shoemaker, R.M. Ottenbrite, Eds.; ACS Symposium Series #362, ACS: Washington D.C., 1988; pp. 217.
40. Bankert, R.B.; Jou, Y.; Mayers, G.L. *Molecular Immunology*; M.Z. Atassi, C.J. van Oss, D.R. Absolom, Eds.; Marcel Dekker: New York, 1984; pp. 175.
41. Ling, N.R.; Jefferis, R. *Practical Immunoassay*; W.R. Butt, Ed.; Marcel Dekker: New York, 1984; pp. 199.
42. Campbell, A.M. *Monoclonal Antibody Technology*; Elsevier: Amsterdam, 1984; pp. 265.
43. Bolton, A.E. *Immunoassays for the 80s*; A. Voller, A. Bartlett, D. Bidwell, Eds.; University Park Press: Baltimore, 1981; pp. 69.
44. Dakubu, S.; Ekins, R.; Jackson, T.; Marshall, N.J. *Practical Immunoassay*; W.R. Butt, Ed.; Marcel Decker: New York, 1984; pp. 71.
45. Bolton, A.E. *Radioiodination Techiques*; Review #18; Amersham Corp.: Arlington Heights, IL, 1978; pp. 71.
46. Walker, W.H.C. *Clin. Chem.* **1977**, *23*, 384.
47. Ekins, R.P.; Chu, F.W. Multianalyte microspot immunoassay-microanalytical "compact disk" of the future, *Clin. Chem.* **1991**, *37*, 1955.
48. Jeffcoate, S.L.; Edwards, R.; Gilby, E.D.; White, N. *Steroid Immunoassay; Proc. 5th Tenovus Workshop*; E.H.D. Cameron, S.G. Hillier, K. Griffiths, Eds.; Alpha Omega Publishing: Cardiff, 1975; pp. 133.
49. Hunter, W.M.; Nars, P.W.; Rutherford, F.J. *Steroid Immunoassay; Proc. 5th Tenovus Workshop*; E.H.D. Cameron, S.G. Hillier, K. Griffiths, Eds.; Alpha Omega Publishing: Cardiff, 1975; pp. 141.
50. Butt, W.R. *Practical Immunoassay*; W.R. Butt, Ed.; Marcel Dekker: New York, 1984; pp. 19.
51. Hawker, C.D. *Anal. Chem.* **1973**, *45*, 878A.
52. Cameron, E.H.D.; Scarisbrick, J.J.; Morris, S.E.; Read, G. *Steroid Immunoassay, 5th Tenovus Workshop*; E.H.D. Cameron, S.G. Hillier, K. Griffiths, Eds.; Alpha Omega Publishing: Cardiff, 1975; pp. 153.
53. Ekins, R.P. *Steroid Immunoassay, Proc. 5th Tenovus Workshop*; E.H.D. Cameron, S.G. Hillier, K. Griffiths, Eds.; Alpha Omega Publishing: Cardiff, 1975; pp. 172.

54. Nordblom, G.D.; Webb, R.; Counsell, R.E.; England, B.G. *Steroids.* **1981**, *38*, 161.
55. Swinkels, L.M.J.W.; Ross, H.A.; Benraad, Th. J. *Ann. Clin. Biochem.* **1991**, *28*, 87.
56. Chard, T. *Methods in Enzymology (70) Immunochemical Techniques*; H. Van Vunakis, J.J. Langone, Eds.; Academic Press: New York, 1980; pp. 280.
57. Collins, W.P.; Barnard, G.J.R.; Hennam, J.F. *Steroid Immunoassay, Proc. 5th Tenovus Workship*; E.H.D. Cameron, S.G. Hillier, K. Griffiths, Eds.; Alpha Omega Publishing: Cardiff, 1975; pp. 223.
58. Odell, W.D. *Methods in Enzymology, (70) Immunochemical Techniques*; H. Van Vunakis, J.J. Langone, Eds.; Academic Press: New York, 1980; pp. 274.
59. A.R. Midgley, Jr.; Hepburn, M.R. *Methods in Enzymology, (70) Immunochemical Techniques*; H. Van Vunakis, J.J. Longone, Eds.; Academic Press: New York, 1980; pp. 266.
60. Odell, W.D.; Silver, D.; Grover, P.K. *Steroid Immunoassays, Proc. 5th Tenovus Workshop*; E.H.D. Cameron, S.G. Hillier, K. Griffiths, Eds.; Alpha Omega Publishing: Cardiff, 1975; pp. 207.
61. Ekins, R.; Chu, F.; Biggart, E. *Clin. Chimica Acta.* **1990**, *194*, 91.
62. Porstmann, T.; Kiessig, S.T. *J. Immunol. Methods.* **1992**, *150*, 5.
63. Engvall, E.; Perlmann, P. *Immunochemistry.* **1971**, *8*, 871.
64. Van Weeman, B.K.; Schuurs, A.H.W.M. *FEBS Lett.* **1971**, *15*, 232.
65. Monroe, D. *Anal. Chem.* **1984**, *56*, 920A.
66. Wisdom, G.B. *Clin. Chem.* **1976**, *22*, 1243.
67. O'Sullivan, M.J. *Practical Immunoassay*; W.R. Butt, Ed.; Marcel Dekker: New York, 1984; pp. 37.
68. Ishikawa, E.; Hashida, S; Kohno, T.; Hirota, K. *Clinica Chim. Acta.* **1990**, *194*, 51.
69. Sauer, M.J.; Foulkes, J.A.; Morris, B.A. *Immunoassays in Food Analysis*; B.A. Morris, M.N. Clifford, Eds.; Elsevier: London, 1985; pp. 53.
70. Allen, J.C.; Smith, C.J. *Tibtech.* **1987**, *5*, 193.
71. Nilsson, B. *Curr. Opinion Immunol.* **1990**, *2*, 898.
72. Chu, F.S. *J. Food Protect.* **1984**, *47*, 562.
73. Avrameas, S. *Immunoassays for the 80s*; A. Voller, A. Bartlett, D. Bidwell, Eds.; University Park Press: Baltimore, 1981; pp. 85.
74. Bannister, J.V.; Higgins, I.J.; Turner, A.P.F. *Biosensor Principles and Applic.* **1991**, *15*, 47.
75. Russell, A.J.; Trudel, L.J.; Skipper, P.L., Groopman, J.D.; Tannenbaum, S.R.; Klibanov, A.M. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 80.
76. Walls, K.W. *Molecular Immunology*; M.Z. Atassi, C.J. Van Oss, D.R. Absolom, Eds.; Marcel Dekker: New York, 1984; pp. 427.
77. Harrison, R. O.; Gee, S.J.; Hammock, B.D. *Biotechnology for Crop Protection*; P.A. Hedin, J.J. Menn, R.M. Hollingworth, Eds.; ACS Symposium Series #379, ACS: Washington D.C., 1988; pp. 316.
78. Jung, F.; Gee, S.J.; Harrison, R.O.; Goodrow, M.H.; Karu, A.E.; Li, Q.X.; Braun, A.L.; Hammock, B.D. *Pestic. Sci.* **1989**, *26*, 303.
79. Morgan, M.R.A.; McNerney, R.; Chan, H.W.-S. *Immunoassays in Food Analysis*; B.A. Morris, M.N. Clifford, Eds.; Elsevier: London, 1985; pp. 159.
80. Landon, J.; Kamel, R.S. *Immunoassays for the 80s*; A. Voller, A. Bartlett, D. Bidwell, Eds.; University Park Press: Baltimore, 1981; pp. 91.
81. Diamandis, E.P.; Christopoulos, T.K. **Anal. Chem. 1990**, *62*, 1149A.
82. Ekins, R.P.; Chu, F.W. *Fresnius J. Anal. Chem.* **1992**, *343*, 23.
83. Olsson, T; Thore, A *Immunoassasys for the 80s*; A. Voller, A. Bartlett, D. Bidwell, Eds.; University Park Press: Baltimore, 1981; pp. 113.
84. Weeks, I.; Campbell, A.K.; Woodhead, J.S.; McCapra, F. *Practical Immunoassay*; W.R. Butt, Ed.; Marcel Dekker: New York, 1984; pp. 103.
85. Hammock, B.D.; Gee, S.J.; Cheung, P.Y.K.; Miyamoto, T.; Goodrow, M.H.; Van Emon, J.; Seiber, J.N. *Pesticide Science and Biotechnology*; R. Greenhalgh, T.R. Roberts, Eds.; Blackwell Scientific: Ottawa, 1987; pp. 309.
86. Ercegovich, C.D. *Pesticide Identification at the Residue Level*; R.F. Gould, Ed.; Advances in Chemistry Series #104, ACS: Washington, D.C., 1971; pp. 162.
87. Hemingway, R.J.; Aharonson, N.; Greve, P.A.; Roberts, T.R.; Thier, H.P. *Pure Appl. Chem.* **1984**, *56*, 1131.
88. Newsome, W.H. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 919.
89. Tye, R.J.; Waite, W.M. *Water Pollut. Control.* **1981**, *80*, 600.
90. Cairns, J.; Mount, D.I. *Environ. Sci. Technol.* **1990**, *24*, 154.
91. Bedding, N.D.; McIntyre, A.E.; Perry, R.; Lester, J.N. *Sci. Total Environ.* **1982**, *25*, 143.
92. Belluck, D.A.; Benjamin, S.L. *J. Environ. Health.* **1990**, *53*, 11.
93. Feliciano, D.V. *J. Water Pollut. Con. Fed.* **1980**, *52*, 1857.
94. Stara, J.F.; Kello, D.; Durkin, P. *Environ. Health Perspec.* **1980**, *34*, 145.
95. Ames, B.N.; Gold, L.S. *Science.* **1989**, *244*, 755.
96. Neal, R.A. *JAWWA.* **1990**, *82*, 44.
97. Ames, B.N.: Gold, L.S. *Science.* **1990**, *250*, 1645.
98. Ames, B.N. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 1.
99. Lijinsky, W. *Environ. Res.* **1989**, *50*, 207.
100. Stevens, J.B.; Swackhamer, D.L. *Environ. Sci. Technol.* **1989**, *23*, 1180.
101. Wegman, R.C.C.; Melis, P.H.A.M. *Crit. Rev. Anal. Chem.*, **1986**, *16*, 281.
102. D'Elia, C.F.; Sanders, J.G.; Capone, D.G. *Environ. Sci. Technol.* **1989**, *23*, 768.
103. MacCarthy, P; Klusman, R.W.; Rice, J.A. *Anal. Chem.* **1989**, *61*, 269R.
104. Frehse, H. *Pesticide Science and Biotechnology*; R. Greenhalgh, T.R. Roberts, Eds.; Blackwell: Oxford, 1987; pp. 293.
105. McCann, J.; Choi, E.; Yamasaki, E.; Ames, B.N. *Proc. Nat. Acad. Sci. U.S.A.* **1975**, *72*, 5135.

106. Trevors, J.T. *Toxicity Testing Using Microorganisms, Vol. 1*; G. Bitton, B.J. Dutka, Eds.; CRC Press: Boca Raton, FL, 1986; pp. 9.
107. Lijinsky, W. *Environ. Carcin. Revs. (J. Envir. Sci. Hlth.)*. **1990**, *C8(1)*, 45.
108. Auletta, A. *Environ. Carcin. Revs. (J. Envir. Sci. Hlth.)*. **1990**, *C8(1)*, 1.
109. Meier, J.R.; Daniel, F.B. *JAWWA*. **1990**, *82*, 48.
110. Mason, J.M.; Langenbach, R.; Shelby, M.D.: Zieger, E.; Tennant, R.W. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 149.
111. Kinae, N.; Hahizume, T.; Makita, T.; Tomita, I.; Kimura, I.; Kanamori, H. *Water Res.* **1981**, *15*, 17.
112. Hooftman, R.N.; Vink, G.J. *Ecotoxicol. Environ. Safety*. **1981**, *5*, 261.
113. Klekowski, E.; Levin, D.E. *Environ. Mutagen.* **1979**, *1*, 209.
114. Cairns, J. Jr.; van der Schalie, W.H. *Water Res.* **1980**, *14*, 1179.
115. Bitton, G.; Koopman, B. *Toxicity Testing Using Microorganisms*; G. Bitton, B.J. Dutka, Eds.; CRC Press: Boca Raton, FL, 1986; pp. 27.
116. Dutka, B.J.; Jones, K.; Kwan, K.K.; McInnis, R. *Water Res.* **1988**, *22*, 503.
117. Burkhard, L.P.; Ankley, G.T. *Environ. Sci. Technol.* **1989**, *23*, 1438.
118. Samolloff, M.R.; Bell, J.; Birkholz, D.A.; Webster, G.R.B.; Arnott, E.G.; Pulak, R.; Madrid, A. *Environ. Sci. Technol.* **1983**, *17*, 329.
119. Parodi, S.; Malacarne, D.; Romano, P.; Taningher, M. *Environ. Health Perspect.* **1991**, *95*, 199.
120. Centeno, E.R.; Johnson, W.J.; Sehon, A.H. *Int. Arch. Allergy*. **1970**, *37*, 1.
121. Haas, G.J.; Guardia, E.J. *Soc. Exper. Biol. Med.* **1968**, *129*, 546.
122. Langone, J.L.; Van Vunakis, H. *Res. Commun. Chem. Pathol. Pharmacol.* **1975** *10*, 163.
123. Lukens, H.R.; Williams, C.B.; Levison, S.A.; Dandliker, W.B.; Murayama, D.; Baron, R.L. *Environ. Sci. Technol.* **1977**, *11*, 292.
124. Luster, M.I.; Albro, P.W.; Chae, K.; Lawson, L.D.; Corbett, J.D.; McKinney, J.D. *Anal. Chem.* **1980**, *52*, 1497.
125. Luster, M.I.; Albro, P.W.; Clark, G.; Chae, K.; Chaudhary, S.K.; Lawson, L.D.; Corbett, J.T.; McKinney, J.D. *Toxicol. Appl. Pharmacol.* **1979**, *50*, 147.
126. McKinney, J.D.; Albro, P.W.; Cox, R.H.; Hass, J.R.; Walters, D.B. *The Pesticide Chemist and Modern Toxicology*; S.K. Bandel, G.J. Marco, L. Goldberg, M.L. Long, Eds.; ACS Symposium Series, ACS: Washington, D.C., 1981; Chapter 25.
127. Schwalbe-Fehl, M. *Int. J. Environ. Anal. Chem.* **1986**, *26*, 295.
128. Mumma, R.O.; Brady, J. *Pesticide Science and Biotechnology*; R. Greenhalgh, T.R. Roberts, Eds.; Blackwell Scientific: Ottawa, 1986; 341.
129. Hammock, B.D. *Biotechnology for Crop Protection*; P.A. Hedin, J.J. Menn, R.M. Hollingworth, Eds.; ACS Symposium Series #379, ACS: Washington D.C., 1988; 298.
130. Vanderlaan, M.; Watkins, B.E.; Stanker, L. *Environ. Sci. Technol.* **1988**, *22*, 247.
131. Fukal, L; Rauch, P.; Kas, J. *Chem. Listy*. **1988**, *82*, 959.
132. Haberer, K.; Kramer, P. *Vom Wasser*. **1988**, *71*, 231.
133. Kaufman, B.M.; Clower, M., Jr. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 239.
134. Wing, K.D.; Hammock, B.D. *Cal. Agric.* **1980**, *34*, 34.
135. Seiber, J.N. *The Impact of Chemistry on Biotechnology*; M. Phillips, S.P. Shoemaker, R.D. Middlekauf, Eds.; ACS Symposium Series #362, ACS: Washington D.C., 1988, 204.
136. Hock, B. *Z. Wasser Abwasser Forsch.* **1989**, *22*, 78.
137. Wraith, M.J.; Britton, D.W. *Brighton Crop Protection Conference-Pests and Diseases (1)*; British Crop Protection Council: London, 1988; 131.
138. Anon. *Pesticide Residues in Foods: Technologies for Detection*; Congress of the U.S.A., Office of Technology Assessment, Washington D.C., 1988, 37.
139. Kimbrough, R.D. *J. Toxicol. Environ. Health*. **1990**, *30*, 261.
140. Safe, S. *Annu. Rev. Pharmacol. Toxicol.* **1986**, *26*, 371.
141. Wolfe, W.H.; Miner, J.C.; Michalek, J.E. An epidemiologic investigation of health effects in air force personnel following exposure to herbicides: serum dioxin analysis of 1987 examination results; Paper presented at Dioxin '91; Raleigh-Durham, NC, Sept. 23–27, 1991; Abstract S77.
142. Fingerhut, M.A.; Halperin, W.E.; Marlow, D.A.; Piacitelli, L.A.; Greife, A.L.; Honchar, P.A.; Sweeny, M.H.; Dill, P.A.; Steenland, N.K.; Suruda, A.J. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; paper presented at Dioxin '91; Raleigh-Durham, NC, September 23–27, 1991; Abstract S78.
143. Johnson, E.S. *Crit. Rev. Toxicol.* **1992**, *21*, 451.
144. McKinney, J.; Albro, P.; Luster, M.; Corbett, B.; Schroeder, J.; Lawson, L. *Chlorinated Dioxins and Related Compounds*; O. Hutzinger et al., Eds.; Pergamon Press: Oxford, 1982; 67.
145. Albro, P.W.; Luster, M.I.; Chae, K.; Clark, G.; McKinney, J.D. *Meth. Enzymol.* **1982**, *84*, 619.
146. Sherry, J.P.; ApSimon, J.W.; Collier, T.L.; Albro, P.W. *Chemosphere*. **1990**, *20*, 1409.
147. Sherry, J.P.; ApSimon, J.; Collier, T.; Afghan, B.; Albro, P. *Chemosphere*. **1989**, *19*, 255.
148. Collier, T.L.; ApSimon, J.W.; Sherry, J.P. *Chemosphere*. **1990** *20*, 301.
149. Collier, L. Synthesis of Dioxins as Analytical and Toxicological Standards, Ph.D. Thesis, Carlton University, Ottawa, Canada; 1989.
150. Sherry, P.; Albro, P.W. Radioimmunoassay of chlorinated dioxins: use of [3H]-labelled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as radioligand; paper presented at Dioxin '90; Bayreuth, Germany, September 10–14, 1990.

151. Kennel, S.J.; Jason, C.; Albro, P.W.; Mason, G.; Safe, S.H. *Toxicol. Appl. Pharmacol.* **1986**, *82*, 256.
152. Stanker, L.; Watkins, B.; Vanderlaan, M.; Budde, W.L.; *Chemosphere.* **1987**, *16*, 1635.
153. Stanker, L.H.; Watkins, B.; Rogers, N.; Vanderlaan, M. *Toxicology.* **1987**, *45*, 229.
154. Vanderlaan, M.; Van Emon, J.; Watkins, B.; Stanker, L.*Pesticide Science and Biotechnology*; R. Greenhalgh, T.R. Roberts, Eds.; Blackwell Scientific: Ottawa, 1987; 597.
155. Vanderlaan, M.; Stanker, L.H.; Watkins, B.E.; Petrovic, P.; Gorbach, S. *Environ. Toxicol. Chem.* **1988**, *7*, 859.
156. Safe, S. *Crit. Rev. Toxicol.* **1984**, *13*, 319.
157. IARC Monograph #18, Lyon 1978; pp. 43; cited in Reference 107.
158. Silberhorn, E.M.; Glauert, H.P.; Robertson, L.W. *Crit.Rev. Toxicol.* **1990**, *20*, 439.
159. Abelson, P.H. *Science.* **1991**, *253*, 361.
160. Newsome, W.H.; Shields, J.B. *Int. J. Environ. Anal. Chem.* **1981**, *10*, 295.
161. Chamerlik-Cooper, M.A.; Carlson, R.E.; Harrison, R.O. Determination of PCBs in soil by enzyme immunoassay; paper presented at *Second International Symposium, Field Screening Methods for Hazardous Waster Site Investigations,* U.S. EPA, Las Vegas, February 1991.
162. Johnson, H.J.; Cemosek, S.F.; Gutierrez-Cemosek, R.M.; Brown, L.L. *J. Anal. Toxicol.* **1980**, *4*, 86.
163. Johnson, H.J.; Cernosek, S.F.; Gutierrez-Cernosek, R.M.; Brown, L.L. *J. Anal. Toxicol.* **1981**, *5*, 157.
164. Gomes, M.; Santella, R. *Chem. Res. Toxicol.* **1990**, *3*, 307.
165. Scheepers, P.T.J.; Beenakkers, M.F.M.; Bos, R.P. *Fres. J. Anal. Chem.* **1992**, *343*, 169.
166. Leginus, J. Westinghouse Bio-analytic Systems Company; fact sheet describing pentachlorophenol immunoassay's performance, personal communication, 1989.
167. Keuchel, C.; Weil, L.; Niessner, R. *Anal. Sci.* **1992**, *8*, 9.
168. Keuchel, C.; Weil, L; Niessner, R. *Fres. J. Anal. Chem.* **1992**, *343*, 143.
169. Li, Q.X.; Zhao, M.S.; Gee, S.J.; Kurth, M.J.; Seiber, J.N.; Hammock, B.D. *J. Agric. Food Chem.* **1991**, *39*, 1685.
170. Rinder, D.F.; Fleeker, J.R. *Bull. Environ. Contam. Toxicol.* **1981**, *26*. 375.
171. Fleeker, J. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 874.
172. Knopp, D.; Nuhn, P.; Dobberkau, H.-J. *Arch. Toxicol.* **1985**, *58*, 27.
173. Knopp, D.; Nuhn, P.; Mittag, E. *Pharmazie.* **1986**, *41*, 143.
174. Hall, J.C.; Deschamps, R.J.A.; Kreig, K.K. *J. Agric. Food Chem.* **1989**, *37*, 981.
175. Dunbar, B.D.; Niswender, G.D.; Hudson, J.M. Antibody for the detection and quantification of atrazine, U.S. Patent #4,530,786, 1985; pp. 6.
176. Huber, S.J. *Chemosphere.* **1985**, *14*, 1795.
177. Dunbar, B; Riggle, B.; Niswender, G. *J. Agric. Food Chem.* **1990**, *38*, 433.
178. Huber, S.J.; Hock, B. *Methods of Enzymatic Analysis*; J. Bergmeyer, M. Grabl, Eds.; VCH Verlagsgesellschaft: Weinheim (Ger), 1986; 438.
179. Sharp, J.K.; Robotti, K.M.; Ehrmann, P.R.; Brown, L.J.; Hermann, B.W. Quantitative analysis of cyanazine in groundwater and soil by enzyme-linked immunoassay; cited by Hermann Reference 37; 1986.
180. Robotti, K.M.; Sharp, J.K.; Ehrmann, P.R.; Brown, L.J.; Hermann, B.W. An ELISA method for the detection of cyanazine, in *Abstracts of Papers Presented at 192nd ACS National Meeting*; ACS: Washington D.C., 1986; AGRO 42.
181. Huber, S.J; Hock, B. *J. Plant Dis. Protect.* **1985**, *92*, 147.
182. Huber, S.J; Hock, B. *GIT Fachz. Lab.* **1985**, *10*, 969.
183. Wittmann, C.; Hock, B. *Food Agric. Immunol.* **1989**, *1*, 211.
184. Wuest, S.; Doht, U.; Giersch, T.; Wittmann, C.; Hock, B. *GIT Fachz. Lab.* **1990**, *34*, 99.
185. Wittmann, C.; Hock, B. *J. Agric. Food. Chem.* **1991**, *39*, 1194.
186. Schneider, R.J.; Weil, L.; Niessner, R. *J. Environ. Anal. Chem.* **1992**, *46*, 129.
187. Aherne, G.W. *Anal. Proc.* **1990**, *27*, 100.
188. Hardcastle, A.; Aherne, W.; Marks, V. *J. Biolumin. Chemilumin.* **1988**, 2, 209.
189. Seare, N.J.; Miller, J.N.; Cole, E.R.; Samuel, A.J.; Woodbridge, A.P. *Anal. Proc.* **1986**, *23*, 220.
190. Valdes, R., Jr.; Miller, J.J. *J. Clin. Immunoassay.* **1992**, *15*, 87.
191. Ulrich, P.; Niessner, R. *Fres. Environ. Bull.* **1992**, *1*, 22.
192. Ulrich, P.; Weil, L.; Niessner, R. *Fres. J. Anal. Chem.* **1992**, *343*, 50.
193. Weller, M.G.; Weil, L.; Niessner, R. *Fres. J. Anal. Chem.***1992**, *343*, 51.
194. Bushway, R.J.; Perkins, B.; Savage, S.A.; Lekousi, S.J.; Ferguson, B.S. *Bull. Environ. Contam. Toxicol.* **1988**, *40*, 647.
195. Schulze, P.A.; Capel, P.D. Utility and limitations of immunoassay tests as a field screening method for triazine herbicides; paper presented at *U.S. Geological Survey's 2nd National Symposium On Water Quality*; Orlando, FL, Nov. 12–17, 1989.
196. Schlaeppi, J.-M.; Fory, W.; Ramsteiner, K. *J. Agric. Food Chem.* **1989**, *37*, 1532.
197. Giersch, T.; Hock, B. *Food Agric. Immunol.* **1990**, *2*, 85.
198. Goodrow, M.H.; Harrison, R.O.; Hammock, B.D. *J. Agric. Food Chem.* **1990**, *38*, 990.
199. Harrison, R.O.; Goodrow, M.H; Hammock, B.D. *J. Agric. Food Chem.* **1991**, *39*, 122.
200. Schneider, P.; Hammock, B.D. *J. Agric. Food Chem.* **1992**, *40*, 525.
201. Levitt, T. *Lancet.* **1977**, *2*, 358.
202. Levitt, T. *Proc. Anal. Div. Chem. Soc.* **1979**, *16*, 72.
203. Fatori, D.; Hunter, W.M. *Clin. Chim. Acta.* **1980**, *100*, 81.

204. Niewola, Z.; Walsh, S.T.; Davies, G.E. *Int. J. Immunopharmacol.* **1983**, *5*, 211.
205. Niewola, Z.; Hayward, C.; Symington, B.A.; Robson, R.T. *Clin. Chim. Acta.* **1985**, *148*, 149.
206. Benner, J.; Niewola, Z. *Methods of Enzymatic Analysis*; J. Bergmeyer, M. Grabl, Eds.; VCH Verlagsgesellschaft: Weinheim, Germany, 1986; pp. 451.
207. Horstmann, B.J.; Chase, H.A.; Kenney, C.N. *J. Chromatogr.* **1990**, *516*, 433.
208. Nagao, M.; Takatori, T.; Wu, B.; Terazawa, K.; Gotouda, H.; Akabane, H. *J. Immunoassay.* **1989**, *10*, 1.
209. Johnston, S.C.; Bowles, M.; Winzor, D.J.; Pond, S.M. *Fund. Appl. Toxicol.* **1988**, *11*, 261.
210. Van Emon, J.M; Sieber, J.N; Hammock, B.D. *Bioregulators for Pest Control*; P.A. Hedin, Ed.; ACS Symposium Series #276, ACS: Washington D.C., 1985; pp. 307.
211. Van Emon, J; Hammock, B.D; Sieber, J.N. *Anal. Chem.* **1986**, *58*, 1866.
212. Van Emon, J.; Sieber, J.; Hammock, B. *Bull. Environ. Contam. Toxicol.* **1987**, *39*, 490.
213. Coxon, R.E.; Rae, C.; Gallacher, G.; Landon, J. *Clin. Chim. Acta.* **1988**, *175*, 297.
214. Schwalbe, M.; Dorn, E.; Beyermann, K. *J. Agric. Food Chem.* **1984**, *32*, 734.
215. Koppatschek, F.K.; Liebl, R.A.; Kriz, A.L; Melhado, L.L. *J. Agric. Food Chem.* **1990**, *38*, 1519.
216. Dargar, R.V.; Tymonko, J.M; VanDerWerf, P. *J. Agric. Food Chem.* **1991**, *39*, 813.
217. Feng, P.C.C.; Wratten, S.F.; Horton, S.R.; Sharp, C.R.; Logusch, E.W. *J. Agric. Food Chem.* **1990**, *38*, 159.
218. Feng, P.C.C.; Horton, S.R.; Sharp, C.R. *J. Agric. Food Chem.* **1992**, *40*, 211.
219. Lawruk, T.S.; Hottenstein, C.S.; Herzog, D.P.; Rubio, F.M. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 643.
220. Itak, J.A.; Selisker, M.Y.; Herzog, D.P. *Chemosphere.* **1992**, *24*, 11.
221. Schlaeppi, J.-M.; Moser, H.; Ramsteiner, K. *J. Agric. Food Chem.* **1991**, *39*, 1533.
222. Scholz, H.M.; Hock, B. *Anal. Lett.* **1991**, *24*, 413.
223. Harrison, R.O.; Brimfield, A.A.; Nelson, J.O. *J. Agric. Food Chem.* **1989**, *37*, 958.
224. Harrison, R.O.; Nelson, J.O. *Food Chem.* **1990**, *38*, 221.
225. Kelley, M.M.; Zahnow, E.W.; Petersen, W.C.; Toy, S.T. *J. Agric. Food Chem.* **1985**, *33*, 962.
226. Schlaeppi, J.M.A.; Meyer, W.; Ramsteiner, K.A. *J. Agric. Food Chem.* **1992**, *40*, 1093.
227. Newsome, W.H.; Collins, P.G. *Food Agric. Immunol.* **1990**, *2*, 75.
228. Kreibg, S.; Hock, B.; Stocker, R. *Anal. Lett.* **1991**, *24*, 1729.
229. Gee, S.J.; Miyamoto, T.; Goodrow, M.H.; Buster, D.; Hammock, B.D. *J. Agric. Food Chem.* **1988**, *36*, 863.
230. Deschamps, R.J.A.; Hall, J.C.; McDermott, M.R. *J. Agric. Food Chem.* **1990**, *38*, 1881.
231. Jung, F.; Szekacs, A.; Li, Q.; Hammock, B.D. *J. Agric. Food Chem.* **1991**, *39*, 129.
232. Newsome, W.H.; Collins, P.G. *Bull. Environ. Contam. Toxicol.* **1991**, *47*, 211.
233. Wong, R.B.; Ahmed, Z.H. *J. Agric. Food Chem.* **1992**, *40*, 811.
234. Li, Q.X.; Hammock, B.D.; Sieber, J.N. *J. Agric. Food Chem.* **1991**, *39*, 1537.
235. Riggle, B. *Bull. Environ. Contam. Toxicol.* **1991**, *46*, 404.
236. Riggle, B.; Dunbar, B.D. *J. Agric. Food Chem.* **1990**, *38*, 1922.
237. Bushway, R.J.; Pask, W.M.; King, J.; Perkins, B.; Ferguson, B.S. *Proceedings of First International Symposium on Field Screening Methods for Hazardous Waste Site Investigations*; Las Vegas, Oct. 11–13, 1988; pp. 433.
238. Dreher, R.M.; Podratzki, B. *J. Agric. Food Chem.* **1988**, *36*, 1072.
239. Frevert, J.; Zietz, E.; Knoell, H.E. *Brighton Crop Protection Conference—Pests and Diseases*; British Crop Protection Council, 1988; pp. 727.
240. Stanker, L.H.; Bigbee, C.; Van Emon, J; Watkins, B.; Jensen, R.H.; Morris, C.; Vanderlaan, M. *J. Agric. Food. Chem.* **1989**, *37*, 834.
241. Wing, K.D.; Hammock, B.D.; Wustner, D.A. *J. Agric. Food Chem.* **1978**, *26*, 1328.
242. Wing, K.D.; Hammock, B.D. *Experientia.* **1979**, *35*, 1619.
243. Van Emon, J.M.; Sieber, J.N.; Hammock, B.D. Application and selectivity of an enzyme-linked immunosorbent assay (ELISA) for the pyrethroid s-bioallethrin; paper presented at *Sixth Int. Congress of Pesticide Chemistry*; Ottawa, 1986; Abstract 5C-01.
244. Roberts, T.R. *Trends Anal. Chem.* **1985**, *4*, 3.
245. Wraith, M.J.; Hitchings, E.J.; Woodbridge, A.P.; Cole, E.R.; Roberts, T.R. Development of immunoassay methods for pyrethroid insecticides; paper presented at *Sixth International Congress of Pesticide Chemistry*; Ottawa, 1986; Abstract 5C-10.
246. Wie, S.I.; Sylwester, A.P.; Wing, K.D; Hammock, B.D. *J. Agric. Food Chem.* **1982**, *30*, 943.
247. Wie, S.I; Hammock, B.D. *J. Agric. Food Chem.* **1982**, *30*, 949.
248. Wie, S.I.; Hammock, B.D. *J. Agric. Food Chem.* **1984**, *32*, 1294.
249. Ercegovich, C.D.; Vallejo, R.P.; Gettig, R.R.; Woods, L.; Bogus, E.R.; Mumma, R.O. *J. Agric Food Chem.* **1981**, *29*, 559.
250. Vallejo, R.P.; Bogus, E.R.; Mumma, R.O. *J. Agric. Food Chem.* **1982**, *30*, 572.
251. Lober, M.; Krantz, S.; Herrmann, I. *Acta Biol. Med. Germ.* **1982**, *41*, 487.
252. Hunter, K.W.; Lenz, D.E. *Life Sci.* **1982**, *30*, 355.
253. Hunter, K.W.; Lenz, D.E.; Brimfield, A.A. *Methods of Enzymatic Analysis*; J. Bergmeyer, M. Grabl, Eds.; VCH Verlagsgesellschaft: Weinheim, Germany, 1986; pp. 426.
254. Brimfield, A.A.; Lenz, D.E.; Graham, C.; Hunter, K.W. *J. Agric. Food Chem.* **1985**, *33*, 1237.
255. Heldman, E.; Balan, A.; Horowitz, O.; Ben-Zion, S.; Torten, M. *FEBS.* **1985**, *180*, 243.

256. Sudi, J.; Heeschen, W. *Kieler Milchwirtschaftlich Forsch.* **1988**, *40*, 179.
257. Duquette, P.H.; Guire, P.E.; Swanson, M.J. Fieldable enzyme immunoassay kits for pesticides; paper presented at *First International Symposium on Field Screening Methods for Hazardous Waste Site Investigation*; Las Vegas; October 11–13, 1988; pp. 239.
258. Hunter, K.W.; Lenz, D.E.; Brimfield, A.A.; Naylor, J.A. *FEBS Lett.* **1982**, *149*, 147.
259. Lenz, D.E.; Brimfield, A.A.; Hunter, K.W.; Benschop, H.P.; De Jong, L.P.A.; Van Dijk, C.; Clow, T.R. *Fund. Appl. Toxicol.* **1984**, *4*, S156.
260. Schmidt, P.; Kuhlmann, R.; Losch, U. *Z. Naturforsch.* **1988**, *43c*, 167.
261. Erhard, M.H.; Schmidt, P.; Kuhlmann, R.; Losch, U. *Arch. Toxicol.* **1989**, *63*, 462.
262. Erhard, M.H.; Kuhlmann, R.; Szinicz, L.; Losch, U. *Arch. Toxicol.* **1990**, *64*, 580.
263. Erhard, M.H.; Jungling, A.; Brendgen, S.; Kellner, J.; Losch, U. *J. Immunoassay.* **1992**, *13*, 273.
264. Brimfield, A.A.; Hunter, K.W.; Lenz, D.E.; Benschop, H.P., Ban Dijk, C.; De Jong, L.P.A. *Molec. Pharmacol.* **1985**, *28*, 32.
265. Buenafe, A.C.; Rittenberg, M.B. *Mol. Immunol.* **1987**, *24*, 401.
266. Skerritt, J.H.; Hill, A.S.; Beasley, H.L.; Edward, S.L.; Mcadam, D.P. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 519.
267. Banerjee, B.D. *Bull. Environ. Contam. Toxicol.* **1987**, *38*, 798.
268. Brady, J.F.; Fleeker, J.R.; Wilson, R.A.; Mumma, R.O. ACS Symposium Series #382, ACS: Washington, D.C., 1989; pp. 262.
269. Brady, J.F. Ph.D. thesis, Penn. State University, University Park, PA. (Cited in Jung et al., 1989a) 1988.
270. Hill, A.S.; Mei, J.V.; Yin, C.-M.; Ferguson, B.S.; Skerrit, J.H. *J. Agric. Food Chem.* **1991**, *39*, 1882.
271. Mei, J.V.; Yin, Ch.-M.; Carpino, L.A.; Ferguson, B.S. *J. Agric. Food Chem.* **1991**, *39*, 2083.
272. Wie, S.I.; Andrews, R.E., Jr.; Hammock, B.D.; Faust, R.M.; Bulla, L.A., Jr. *Appl. Environ. Microbiol.* **1982**, *43*, 891.
273. Wie, S.I.; Hammock, B.D.; Gill, S.S.; Grate, E.; Andrews, R.E., Jr.; Faust, R.M., Bulla, L.A., Jr.; Schaefer, C.H. *Appl. Environ. Microbiol.* **1984**, *57*, 447.
274. Cheung, P.Y.K.; Hammock, B.D. *Biotechnology for Crop Protection*; P.A. Hedin, J.J. Menn, R.M. Hollingworth, Eds.: ACS Symposium Series # 379, ACS: Washington, D.C., 1988; 359.
275. Smith, R.A.; Ulrich, J.T. *Appl. Environ. Microbiol.* **1983**, *45*, 586.
276. Wikman-Coffelt, J.; Berg, H.W. *Am. J. Enol. Vitic.* **1976**, *27*, 115.
277. Newsome, W.H.; Shields, J.B. *J. Agric. Food Chem.* **1981**, *29*, 220.
278. Newsome, W. H.; Collins. P.G. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 1025.
279. Bushway, R.J.; Savage, S.A.; Ferguson, B.S. *Food Chem.* **1990**, *35*, 51.
280. Newsome, W.H. *J. Agric. Food Chem.* **1985**, *33*, 528.
281. Newsome, W.H. *Bull. Environ. Contam. Toxicol.* **1986**, *36*, 9.
282. Newsome, W.H. *Pesticide Science and Biotechnology*; R. Greenhalgh, T.R. Roberts, Eds.; Blackwell Scientific: Ottawa, 1987; pp. 349.
283. Jung, F.; Meyer, H.H.D.; Hamm, R.T. *J. Agric. Food Chem.* **1989**, *37*, 1183.
284. Meyer, H.H.D. *Nuclear and Related Techniques in Animal Production and Health*; Food and Agricultural Organization of the UN, Int. Atomic Agency: Vienna, 1986; pp. 255.
285. Meyer, H.H.D.; Guven, B. *J. Steroid Biochem.* (Suppl) **1986**, *25*, 139.
286. Forlani, F.; Arnoldi, A.; Pagani, S. *J. Agric. Food Chem.* **1992**, *40*, 328.
287. Kitagawa, T.; Kawasaki, T.; Munecika, H. *J. Biochem.* **1982**, *92*, 585.
288. Chung, S.-Y.; Johnsen, P.B.; Klesius, P.H. *J. Agric. Food Chem.* **1990**, *38*, 410.
289. Chung, S.Y. *Water Sci. Technol.* **1992**, *25*, 89.
290. Wie, S.I.; Hammock, B.D. *Anal. Biochem.* **1982**, *125*, 168.
291. Richardson, M.L.; Bowron, J.M. *J. Pharm. Pharmacol.* **1985**, *37*, 1.
292. Aherne, G.W.; English, J.; Marks, V. *Ecotoxicol. Environ. Safety.* **1985**, *9*, 79.
293. Van de Water, C.; Haagsma, N. *Food Agric. Immunol.* **1990**, *2*, 11.
294. Arnold, D.; Somogyi, A. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 984.
295. Rohner, P.; Schallibaum, M.; Nicolet, J. *J. Food Protect.* **1985**, *48*, 59.
296. Van de Water, C.; Haagsma, N. *J. Chromatogr.* **1991**, *566*, 173.
297. Van de Water, C.; Haagsma, N. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 534.
298. Groopman, J.D.; Trudel, L.J.; Donahue, P.R.; Marshak-Rothstein, A.; Wogan, G.N. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7728.
299. Mortimer, D.N.; Shepherd, M.J.; Gilbert, J.; Morgan, M.R.A. *Food Additives Comtam.* **1987**, *5*, 127.
300. Morgan, M.R.A.; McNerney, R.; Chan, H.W.S. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1481.
301. Morgan, M.R.A.; Kang, A.S.; Chan, H.W.S. *J. Sci. Food Agric.* **1986**, *37*, 908.
302. Duchatel, J.P.; Maghuin-Rogister, G. *Ann. Rech. Vet.* **1985**, *16*, 93.
303. Warner, R.; Ram, B.P.; Hart, P.; Pestka, J.J. *J. Agric. Food Chem.* **1986**, *34*, 714.
304. Teshima, R.; Kawase, M.; Tanaka, T.; Hirai, K.; Sato, M.; Sawada, J.; Ikebuchi, H.; Ichinoe, M.; Terao, T. *J. Agric. Food Chem.* **1990**, *38*, 1618.
305. Liu, M.-T.; Ram, B.P.; Hart, P.; Pestka, J.J. *Appl. Environ. Microbiol.* **1985**, *50*, 332.
306. Wylie, D.E.; Carlson, L.D.; Carlson, R.; Wagner, F.W.; Schuster, S.M. *Anal. Biochem.* **1991**, *194*, 381.

307. Chait, E.M.; Ebersole, R.C. *Anal. Chem.* **1981**, *53*, 682A.
308. Ekins, R.P. *Immunoassays for Clinical Chemistry*; W.M. Hunter, J.E.T. Corrie, Eds.; Churchill Livingstone: Edinburgh, 1983; pp. 76.
309. Gilman, A.P.; Douglas, V.M.; Newhook, R.C.; Arbuckle, T.E. *Chlorophenols and their impurities: A Health Hazard Evaluation*; Health and Welfare Canada: Ottawa, 1988; 88-EHD-110, pp. 72.
310. Sherry, J.P.; Stanker, L.; Watkins, B.; Vanderlaan, M.; Albro, P. Monoclonal antibody based radioimmunoassay for the detection of polychlorinated dibenzo-*p*-dioxins, paper presented at *Dioxin '91*; North Carolina, Sept. 23–27, 1991; Poster #171.
311. Niewola, Z.; Benner, J.P.; Swaine, H. *Analyst.* **1986**, *111*, 399.
312. Anon. *ImmunoTechnology Catalog and Handbook*; Pierce: Rockford, IL, 1990.
313. Kuniyuki, A.H.; McCarthy, S. A novel method for the development of monoclonal antibodies to Surflan; paper presented at *Sixth International Congress of Pesticide Chemistry*; Poster Session, Ottawa, August 11–15, 1986.
314. Wong, J.M.; Li, Q.X.; Hammock, B.D.; Seiber, J.N. *J. Agric. Food Chem.* **1991**, *39*, 1802.
315. Goh, K.S.; Hernandez, J.; Powell, S.J.; Greene, C.D. *Bull. Environ. Contam. Toxicol.* **1990**, *45*, 208.
316. Goh, K.S.; Hernandez, J.; Powell, S.J.; Garretson, C.; Troiano, J.; Ray, M.; Greene, C.D. *Bull. Environ. Contam. Toxicol.* **1991**, *46*, 30.
317. Stearman, G.K.; Adams, V.D. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 144.
318. Bushway, R.J.; Perkins, L.B.; Hurst, H.L.; Ferguson, B.S. *Food Chem.* **1992**, *43*, 283.
319. Thurman, E.M.; Meyer, M.; Pomes, M.; Perry, C.A.; Schwab, A.P. *Anal. Chem.* **1990**, *62*, 2043.
320. Li, Q.X.; Gee, S.J.; McChesney, M.M.; Hammock, B.D.; Seiber, J.N. *Anal. Chem.* **1989**, *61*, 819.
321. Stocklein, W.; Gebbert, A.; Schmidt, R.D. *Anal. Lett.* **1990**, *23*, 1465.
322. Weetal, H.H. *J. Immunol. Methods.* **1991**, *136*, 139.
323. Borman, S. *C&EN News.* **1992**, Jan. 13, 5.
324. Hock, B. *Anal. Lett.* **1991**, *24*, 529.
325. Anon. *Fed. Regist.* **1989**, *54*, 33920.
326. Afghan, B.K.; Carron, J.; Goulden, P.D.; Lawrence, J.; Leger, D.; Onuska, F.; Sherry, J.; Wilkinson, R.J.; *Can. J. Chem.* **1987**, *65*, 1086.
327. Bushway, R.J.; Perkins, L.B.; Fukal, L.; Harrison, R.O.; Ferguson, B.S. *Arch. Environ. Contam. Toxicol.* **1991**, *21*, 365.
328. Bushway, R.J.; Hurst, H.L.; Perkins, L.B.; Tian, L.; Cabanillas, C.G.; Young, B.E.S.; Ferguson, B.S.; Jennings, H.S. *Bull. Environ. Contam. Toxicol.* **1992**, 49, 1.
329. Bushway, R.J.; Perkins, B.; Savage, S.A.; Lekousi, S.L.; Ferguson, B.S. *Bull. Environ. Contam. Toxicol.* **1989**, *42*, 899.
330. Goh, K.S.; Richman, S.J.; Troiano, J.; Garretson, C.L.; Hernandez, J.; Hsu, J.; White, J.; Barry, T.A.; Ray, M.; Tran, D.; and Miller, N.K. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 554.
331. Harrison, R.O.; Braun, A.L.; Gee, S.J.; O'Brien, D.J.; Hammock, B.D. *Food Agric. Immunol.* **1989**, *1*, 37.
332. Tomita, M.; Suzuki, K.; Shikmosato, K.; Kohama, A.; Ijiri, I. *Forensic Sci. Int.* **1988**, *37*, 11.
333. Rang, K.T.; Xu, Q.H.; Shao, R.X.; Gong, X.Q. *Acta Pharmacol. Sin.* **1986**, *1*, 93.
334. Bushway, R.J.; Kugabalasooriar, J.; Perkins, L.B.; Harrison, R.O.; Young, B.E.S.; Ferguson, B.S. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 323.
335. Lee, S.M.; Richman, S. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 893.
336. Ruitenberg, E.J.; van Amstel, J.A.; Brosi, B.J.M.; Steerenberg, P.A. *J. Immunol. Methods.* **1977**, *16*, 351.
337. Ng, R.H. *J. Clin. Immunoassay.* **1991**, *14*, 59.
338. Dudley, R.F. *J. Clin. Immunoassay.* **1991**, *14*, 77.
339. Jefferson, R. *J. Clin. Immunoassay.* **1991**, *14*, 89.
340. Baselt, R.C. *J. Anal. Toxicol.* **1989**, *13*, 1.
341. Van Ginkel, L.A. *J. Chromatogr.* **1991**, *564*, 363.
342. BioNebraska Inc. Information on commercial IA products; personal communication, Moorestown, NJ, 1991.
343. Ohmicron Inc. Information kit on commercial IA products; personal communication, Newtown, PA, 1991.
344. Environmental Diagnostics, Inc. *Annual Report 1989*, Burlington, NC, 1989.
345. Andrews, R. *Scientist.* **1992**, *6*, 17.
346. Knopp, D.; Glass, S. *Int. Arch. Occup. Environ. Health.* **1991**, *63*, 329.
347. Reed, J.P.; Hall, F.R.; Kreuger, H.R. *Bull. Environ. Contam. Toxicol.* **1990**, *44*, 8.
348. Rosner, M.H.; Grassman, J.S.; Haas, R.A. *Environ. Health Perspects.* **1991**, *94*, 131.
349. Goksoyr, A.; Solberg, T.S.; Serigstad, B. *Marine Pollut. Bull.* **1991**, *22*, 122.
350. Walters, R.R. *Anal. Chem.* **1985**, *57*, 1099A.
351. Katz, S.E.; Brady, M.S. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 557.
352. Glencross, R.G.; Abeywardene, S.A.; Corney, S.J.; Morris, H.S. *J. Chromatogr.* **1981**, *223*, 193.
353. Hamers, A.R.M.; Paulussen, R.J.A. The use of antibodies for residue analysis in biological samples, in *Proc. of the Symposium 'Perspectives for Monoclonal Antibodies in Agriculture'*; Laboratory for Monoclonal Antibodies: Wageningen, Netherlands, 1990; pp. 63.
354. Farjam, A.; Vandemerbel, N.C.; Lingeman, H.; Frei, R.W.; Brinkman, U.A.T. *Int. J. Environ. Anal. Chem.* **1991**, *45*, 73.
355. Farjam, A.; Brugman, A.E.; Lingeman, H.; Brinkman, U.A.Th. *Analyst.* **1991**, *116*, 891.
356. Farjam, A.; Vreuls, J.J.; Cuppen, W.J.G.M.; Brinkman, U.A.Th.; De Jong, G.J. *Anal. Chem.* **1991**, *63*, 2481.
357. Davis, G.C.; Hein, M.B.; Chapman, D.A. *J. Chromatogr.* **1986**, *366*, 171.
358. Van de Water, C.; Haagsma, N. *J. Chromatogr.* **1987**, *411*, 415.

359. Mortimer, D.N.; Gilbert, J.; Shepherd, M.J. *J. Chromatogr.* **1987**, *407*, 393.
360. Van de Water, C.; Tebbal, D.; Haagsma, N. *J. Chromatogr.* **1989**, *478*, 205.
361. Van Ginkel, L.A.; Stephany, R.W.; Van Rossum, H.J.; Steinbuch, H.M.; Zomer, G.. Van De Heeft, E.; De Jong, A.P.J.M. *J. Chromatogr.* **1989**, *489*, 111.
362. Schultz, J.S. *Sci. Am.* **1991**, Aug., 64.
363. Guilbault, G.G.; Schmid, R.D. *Biotechnol. Appl. Biochem.* **1991**, *14*, 133.
364. Bright, F.V.; Betts, T.A.; Litwiler, K.S. *Anal. Chem.* **1990**, *62*, 1065.
365. Ahluwalia, A.; De Rossi, D.; Monici, M.; Schirone, A. *Biosensors and Bioelectronics.* **1991**, *6*, 133.
366. Lu, B.; Lu, C.; Wei, Y. *Anal. Lett.* **1992**, *25*, 1.
367. Anis, N.A.; Wright, J.; Rogers, K.R.; Thompson, R.G.; Valdes, J.J.; Eldefrawl, M.E. *Anal. Lett.* **1992**, *25*, 627.
368. Bolts, J.M.; Diamond, S.E.; Kolc, J.F.; Lin, S.H.; Regina, F.J.; Koga, P.G.; Misener, G.C.; Schmidt, J.C. Integrated immunochemical systems for environmental monitoring, in *Proceedings of First International Symposium on Field Screening Methods for Hazardous Waste Site Investigations*; Las Vegas, Oct. 11–13, 1988; pp. 243.
369. Nakamura, N.; Matsunaga, T. *Anal. Lett.* **1991**, *24*, 1075.
370. Stanbro, W.D.; Newman, A.L.; Hunter, K.W. *Biotechnology for Crop Protection*; P.A. Hedin, J.J. Menn, R.M. Hollingworth, Eds.; ACS Symposium Series #379, ACS, Washington, D.C., 1989; pp. 331.
371. Kusterbeck, A.W.; Wemhoff, G.A.; Charles, P.T.; Yeager, D.A.; Brederhorst, R.; Vogel, C.-W.; Ligler, F.S. *J. Immunol. Methods.* **1990**, *135*, 191.
372. Hlady, V.; Lin, J.N.; Andrade, J.D. *Biosensors.* **1990**, *5*, 291.
373. Thompson, M.; Krull, U.J. *Anal. Chem.* **1991**, *63*, 393A.
374. Heineman, W.R.; Halsall, H.B. *Anal. Chem.* **1985**, *57*, 1321A.
375. O'Daly, J.P.; Zhao, J.; Brown, P.A.; Henkens, R.W. *Enzyme Microb. Technol.* **1992**, *14*, 299.
376. Zaitsev, V.N.; Colomiets, L.I.; Elskaya, A.V.; Skopenko, V.V.; Evans, J. *Anal. Chim. Acta.* **1991**, *252*, 1.
377. Schmid, R.D.; Kunnecke, W. *J. Biotechnol.* **1990**, *14*, 3.
378. Kramer, P.; Schmid, R. *Biosensors Bioelectronics.* **1991**, *6*, 239.
379. Pollema, C.H.; Ruzicka, J.; Lernmark, A.; Christian, G.D. *Microchem. J.* **1992**, *45*, 121.
380. Conaway, J.E. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 715.
381. Ekins, R.P.; Chu, F.W.; Biggart, E.M. *J. Clin. Immunoassay.* **1990**, *13*, 169.
382. Kricka, L. J. *Clin. Chem.* **1992**, *38*, 327.
383. Ekins, R.; Chu, F.; Biggart, E. *Anal. Chim. Acta.* **1989**, *227*, 73.
384. Kakabakos, S.E.; Christopoulos, T.K.; Diamandis, E.P. *Clin. Chem.* **1992**, *38*, 338.
385. Maxey, K.M.; Maddipati, K.R.; Birkmeier, J. *J. Clin. Immunoassay.* **1992**, *15*, 116.
386. Anon. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 694.
387. Mastrorocco, D.; Brodsky, M. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 331.
388. Miller, J.J.; Valdes, J., Jr. *J. Clin. Immunoassay.* **1992**, *15*, 97.
389. Avrameas, S. *J. Immunol. Methods.* **1992**, *150*, 23.
390. Rogers, K.R.; Lin, J.N. *Biosensors Bioelectronics.* **1992**, *7*, 317.
391. Van Emon, J.M.; Lopez-Avila, V. *Anal. Chem.* **1992**, *64*, 79A.
392. Van Emon, J.M.; Gerlach, R.W. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 635.