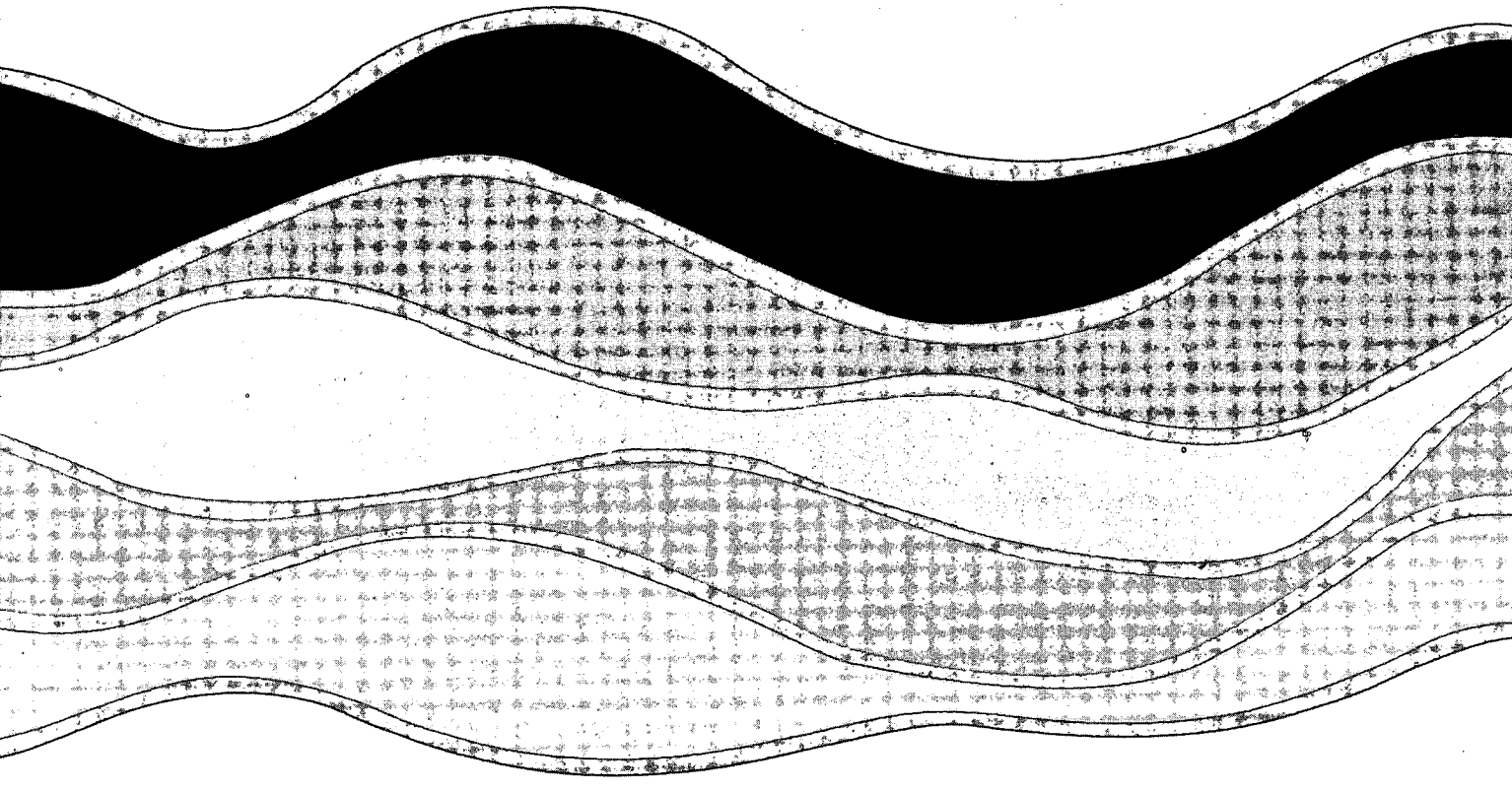
92-03

CCIW
MAY 14 1992
LIBRARY

TIONAL
ATER
RESEARCH
INSTITUTE

INSTITUT
NATIONAL
de RECHERCHE
sur les
EAUX



ENVIRONMENTAL CHEMISTRY: THE IMMUNOASSAY OPTION

J.P. Sherry

NWRI Contribution No. 92-03

TD
226
N87
No. 92-
03
c. 1

ENVIRONMENTAL CHEMISTRY: THE IMMUNOASSAY OPTION

J.P. Sherry

Research and Applications Branch
National Water Research Institute
Burlington, Ontario L7R 4A6

NWRI Contribution No. 92-03

TABLE OF CONTENTS

MANAGEMENT PERSPECTIVE

The surveillance and monitoring of aquatic environments for chemical contaminants can be costly. Many laboratories are barely able to cope with current sample loads, and those loads are likely to increase as a result of regulatory and political pressures. There is a requirement for new and improved analytical methods that will boost productivity and lower costs. At present, valuable resources are used to analyze samples that are either analyte free or have negligible analyte levels. Methods that could screen out such 'negative' samples, preferably after minimal sample preparation, would lower the overall cost of analytical programs. Immunoassay (IA) screening techniques for the detection of a broad variety of pollutants, including pesticides and industrial contaminants, are reviewed. Most methods are sufficiently sensitive and selective for use in monitoring the aquatic environment. In many cases samples can be analyzed with little or no preparation. IAs are best suited to the analysis of large sets of samples. IA kits are commercially available for those without the resources to develop their own assays. IAs could play a key role in the cost-effective and innovative laboratory of the future.

The present review indicates that IAs for the detection of environmental contaminants are gaining wider acceptance. New assays have been developed for many additional pollutants over the past five years. Routine analytical laboratories should explore the use of IA techniques to complement traditional analytical schemes in an attempt to reduce costs and improve productivity.

SOMMAIRE À L'INTENTION DE LA DIRECTION

La surveillance des milieux aquatiques afin de déceler la présence de contaminants chimiques peut être coûteuse. De nombreux laboratoires peuvent à peine traiter le volume actuel des échantillons, et ces volumes sont appelés à augmenter en raison des exigences liées à la réglementation et des pressions au niveau politique. Des méthodes d'analyse nouvelles et améliorées sont nécessaires pour accroître la productivité et abaisser les coûts. À l'heure actuelle, des ressources importantes sont utilisées pour analyser des échantillons qui ne contiennent pas de substances cibles à analyser ou dont les teneurs sont négligeables. Le recours à des méthodes qui pourraient dépister des échantillons "négatifs", de préférence après une préparation minimale de l'échantillon, abaisserait le coût global des programmes d'analyse. Le présent rapport examine des techniques de dosages immunologiques en vue du dépistage d'une vaste gamme de polluants, notamment de pesticides et de contaminants industriels. La plupart des méthodes sont suffisamment sensibles et sélectives pour en permettre l'emploi dans le cadre d'activités de surveillance du milieu aquatique. Dans bon nombre de cas, des échantillons peuvent être analysés sans préparation ou avec peu de préparation. Les dosages immunologiques conviennent mieux à l'analyse de grands ensembles d'échantillons. Les établissements qui ne disposent pas des ressources nécessaires pour élaborer leurs propres essais peuvent se procurer des trousses de dosage immunologique. Les dosages immunologiques pourraient jouer un rôle important au niveau du laboratoire de l'avenir au plan de la rentabilité et de l'innovation.

La présente étude montre que les dosages immunologiques pour le dépistage des contaminants environnementaux sont de plus en plus acceptés. Au cours des cinq dernières années, de nouveaux essais ont été mis au point pour de nombreux autres polluants. Des laboratoires d'analyses courantes devraient étudier la possibilité d'appliquer des techniques de dosage immunologique comme complément aux programmes d'analyse classique pour tenter de réduire les coûts et d'accroître la productivité.

ABSTRACT

Environmental managers and analysts alike are becoming more interested in techniques that will help to lower costs and improve efficiency. Immunoassays (IAs), which have already revolutionised clinical chemistry, could play a key role in the cost-effective and innovative laboratory of the future. IA screening techniques for the detection of a broad variety of pollutants, including pesticides and industrial contaminants, are reviewed. The majority of the methods are sufficiently sensitive and selective for use in monitoring the aquatic environment. In many cases samples can be analyzed with little or no preparation. IAs are best suited to the analysis of large sets of samples. IA kits are commercially available for those without the resources to develop their own assays. Future trends will probably include the use of immunoaffinity probes for the direct detection of contaminants, the use of immunoaffinity chromatography for the one step purification of trace contaminants, and multi-analyte assays that are based on arrays of antibody microspots. Such developments will boost productivity and will also enable laboratory managers to reserve costly instruments, such as mass spectrometers, for the quantification of positive samples.

RÉSUMÉ

Des gestionnaires de l'environnement de même que des analystes sont de plus en plus intéressés par des techniques qui leur permettront d'abaisser les coûts et d'accroître l'efficacité. Des dosages immunologiques, qui ont déjà révolutionné la chimie clinique, pourraient jouer un rôle clé au niveau du laboratoire de l'avenir qui serait rentable et innovateur. Le présent rapport traite des techniques de dosage immunologique pour le dépistage d'une vaste gamme de polluants, notamment de pesticides et de contaminants industriels. La majorité des méthodes sont suffisamment sensibles et sélectives pour permettre leur emploi dans la surveillance du milieu aquatique. Dans bon nombre de cas, des échantillons peuvent être analysés sans préparation ou avec peu de préparation. Les dosages immunologiques conviennent mieux à l'analyse de grands ensembles d'échantillons. Les établissements qui ne disposent pas des ressources nécessaires pour élaborer leurs propres essais peuvent se procurer des trousses de dosage immunologique. Les tendances futures comprendront probablement le recours à des sondes d'immunoaffinité pour le dépistage direct de contaminants, la chromatographie par immunoaffinité pour la purification en une seule étape de contaminants à l'état de trace, et des essais visant l'analyse simultanée de plusieurs substances fondés sur une collection de microtaches d'anticorps. De tels progrès accroîtront la productivité et permettront également aux gestionnaires des laboratoires de réserver l'utilisation d'instruments coûteux, comme des spectromètres de masse, à la quantification d'échantillons positifs.

INTRODUCTION

Modern society has released a myriad man-made chemicals into the environment. This is hardly a surprise since about 500,000 chemicals are in use worldwide (1), and about 76,000 of those chemicals are in daily use (2). The chemical emissions of industry, agriculture, and municipalities often end up in surface or ground water (3, 4). Water is a major transportation vector for chemical pollutants; the oceans and large inland lakes are the ultimate sinks (5). The hundreds of new compounds that enter the market place annually (6) will aggravate the crisis (3) unless remedial action is taken.

In addition to being concerned 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 and remains controversial (3, 4, 7 - 9). One 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 minimise exposure to harmful agents as part of the effort to improve public health (10).

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 must also be assessed. The surveillance and monitoring of aquatic environments for chemical contaminants can be an onerous and costly task. Because humans are exposed to aquatic pollutants mainly by the consumption of contaminated water or biota (11), much attention is focused on those matrices. Analytes that require enrichment and the use of sophisticated detectors can be particularly difficult to determine (12 - 15). 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 (**16**). 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, and 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 (**17, 18**), 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 (**1**). Since many carcinogens are mutagenic in *in-vitro* tests (**17, 19, 20**) the short term mutagenicity tests can be used to rank samples according to carcinogenic potential and potential health risk (**21**). There are many other short term mutagenicity tests (**21, 22**), most of which can be readily adapted to environmental tasks. Mutagenicity (**23**) and cytogenicity tests (**24, 25**) 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 (**26**), batteries of short term biochemical (**27**), genotoxicity, and bioassay tests (**28**) have been used to screen samples (**2**). Positive samples can be fractionated and re-analyzed so as to identify the toxicant (**29, 30**). Unfortunately many carcinogens test negative in the various short term mutagenicity tests (**19**), and genotoxicity tests do not detect carcinogens such as benzene and many of the halogenated aromatic hydrocarbons (**19, 20**).

Immunoassay (IA) screening tests are a blend of techniques from analytical and clinical chemistry (**15, 31**), and are among the most promising techniques for the detection of targeted contaminants. IAs employ the selective and sensitive antibody (AB) - antigen (AG) reaction to detect organic molecules. 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 (**32, 33**). Centeno et al. (**32**) 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 (**34**) extended that concept in a pivotal article that proposed the use of immunological screening methods for the rapid detection of pesticide residues. He foresaw that IAs could be used not only to rapidly screen large sample sets, but also to confirm the results of conventional techniques. A radioimmunoassay (RIA) for the insecticides aldrin and dieldrin (**35**) was the first reported assay for an environmental contaminant. An IA for 2-aminobenzimidazole, a degradation product of the fungicide Benomyl (**36**), and a set of assays for some important chlorinated pollutants (**37 - 39**) soon followed.

Because IAs are ideal for determining the absence of analyte, they can expedite the elimination of "negative" samples from large sample sets. 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 (**40**). The screening out of "negative samples" would be particularly beneficial in the case of trace contaminants that require complex multi-step clean-up procedures and sophisticated instrumental quantification (**41, 42**). IA screening techniques can lower average analytical costs by a factor of five (**31**), and for many analytes could eliminate the need for extensive sample clean-up (**40**). It now costs about $1 billion a year to monitor environmental contaminants in the United States (**31**). Clearly there are considerable savings to be made. The maturing interest in environmental IAs is evident from the reviews (**42 -52, 54 - 56**) and supportive articles (**57 - 62**) that have been published in the past decade. The commercial prospects for environmental IA kits have also been assessed (**31, 47, 49**).

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 in the aquatic environment.

Background

Yalow and Berson (**63**) won the Noble Prize for their development of a quantitative immunological assay of human insulin that made possible the accurate measurement of pg hormone levels in small samples of body fluid. The technique was based on the ability of insulin to displace ^{131}I labelled beef insulin from AB-insulin complexes. The displacement of tracer was proportional to the amount of unlabelled insulin. The evolution of IAs as routine analytical tools ensued. IAs main advantage over other binding assays, is the ability of the immune system to produce, on demand, antibodies to virtually any organic molecule. Three decades after their invention IAs are widely used in clinical laboratories (**66**) because they are sensitive, specific, and cost effective (**43, 65**). IAs have been developed for many analytes including pharmaceuticals, parasites, and bacteria (**66 - 70**).

Antibodies are the essential IA reagent, and they tend more than the other reagents to determine an assay's characteristics. The antibodies used in IAs belong to the immunoglobulin's gamma fraction (IgG) and are produced by mammalian lymphocyte B cells, usually in conjunction with T helper cells (**62, 71 - 74**), as part of the immune systems response to foreign substances (**76, 77**). Each differentiated B cell clone (plasma cells) secretes a single antibody type. Because the divalent ABs are formed against each of the immunogen's antigenic sites, the resultant serum is a mixture (poly-) of clonal antibodies. The weak AB-AG interaction involves only non-covalent bonds: Van der Waals interactions, electrostatic bonds, hydrogen bonds, and hydrophobic bonds. The first two bond types usually predominate. Since AB-AG interactions occur over short distances, a close steric fit combined with a precise match of oppositely charged ions promotes binding (**78**).

Ab-Ag binding depends on the physical and chemical properties of the reactants, and obeys the law of mass action (**43, 64, 79**):

$$Ab + Ag <> AbAg$$

$$K = [AbAg]/[Ab] + [Ag].$$

K is a measure of the serum's avidity for antigen. For some serum - antigen combinations the mass action equation may be an over simplification since it averages many heterogenous interactions (**78**). In competitive IAs, such as RIA, the reagents are usually adjusted so that 30 - 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 (**80**).

Advantages and Limitations of IA Techniques

There is general consensus that the many advantages of the IA technique outweigh its limitations (Table 1). Because IAs are inherently adaptable (**44**) their perceived limitations can often be overcome by creative assay design. Some limitations, such as the tendency to cross react, can be advantageous if the intent is to detect classes of related compounds. Although IAs are unsuitable for small sample sets, their efficiency and cost effectiveness improves as the sample load increases. IAs' have generally low detection limits (1-50 pg) which compare well with the best conventional methods. Such DLs can often be achieved with little or no sample preparation. From many perspectives IAs are a most promising screening technique (**31, 34, 40, 44, 45, 68**). At its present state of development, however, the technology is not suited to multi-residue applications.

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 70000) is a popular carrier (**81**); it has plenty of free NH_2 groups and is easily solubilized. Other proteins can also be effective (Table 2); 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 (58).

Often the target compound contains a functional group that can be covalently coupled to the carrier molecule (Table 2). If not, a suitable group (NH_2, COOH, OH, SH, CO, HCO) can be introduced into the hapten. In either case, care should be taken to avoid undesirable masking of characteristic features (76), which could adversely affect antibody specificity. ABs are usually most specific for parts of the molecule furthest from the site of conjugation (82, 83), and lowest for adjoining sites (70, 84). 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 sub-unit. 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 (43, 76, 85). Good antibody titres have been obtained using hapten conjugation ratios of 8-25 (85) although there is no consensus as to which ratio is best (43, 83, 86). Satisfactory immune responses have been reported for conjugation ratios as low as 2 to 6 haptens per protein molecule (83). It has been suggested that low substitution ratios are less likely to induce the production of low affinity antibodies (83).

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 2). 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 (37, 85, 88). The mixed anhydride reaction does not lead to the formation of excessive cross-links in the protein molecule (100). Haptens that contain carboxyl groups can also be conjugated using the straight forward water soluble carbodiimide (CDI) method (43, 76, 85), or the CDI based N-hydroxysuccinimide (NHS) activated ester reaction (85, 89). The latter method minimises 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 (43). Haptens that contain aromatic NH_2 groups can be diazotized and coupled directly to proteins (76, 91). Hapten NH_2 groups can also be derivatized using an acid anhydride, or an acid chloride of a dicarboxyllic acid and then coupled to protein via the remaining -COOH group. 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 (92). Conjugation methods are available for other functionalities (83).

Antisera

Once purified and characterised, 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 polyclonal ABs (PABs). Because the titre, specificity, and avidity of sera can vary, the chances of a satisfactory immune response are increased if several animals are immunized. PABs are most often raised in rabbits because they are readily available, are inexpensive to maintain, and are usually responsive. The host animal is injected intramuscularly or via multiple intradermal routes with an emulsion of the immunogen in a suitable adjuvant (84, 86, 93). The adjuvant, usually Freund's, slows the immunogen's release from the injection site, and stimulates the immune response (43). When the antibody titre 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 antigen or the linkage arm is immobilized on a support bed; the matching ABs are removed from the serum by passing the serum through the column (37, 48, 93). 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 (**80**).

Monoclonal Antibodies (MABs)

Although most assay formats are not unduly influenced by the AB's clonal type (**42, 86**), many 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 (**94, 95**). 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 antibodies 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 produced in high yields. Detailed descriptions of MAB production techniques are available from other sources (**72, 76, 94 - 96**). The clonal screening process, can be used to enhance an assay's specificity; clones can be selected that ignore the hapten - protein linker arm and are specific for the target molecule. MABs usually bind to a single antigenic determinant. The steep dose-response curves of many MAB based assays contributes to assay precision (**43**). Also, because of the clonal screening process, the immunogen does not need to be highly pure (**43, 86**). MAB technology can be used to standardize all aspects of an IA (**43, 50, 76, 94**). Hybridoma technology is not problem free, however. MABs are costly to produce (**43, 76, 86, 96**); the chromosome complement of the hybrid cells can be unstable (**42, 43**); and assay specificity can be too narrow for some screening tasks.

Immunoassay Types

The main types of immunoassay are differentiated 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.

Radioimmunoassay: Radio-ligands, especially those that emit gamma radiation, can be rapidly, conveniently, and sensitively counted (**92, 98, 99**). Pg level determinations are usually possible using RIA (**66**). Unlike some non-isotopic IAs, RIA's final quantification step is unaffected by non specific interferences (**100, 101**).

^{125}I, which combines high isotopic abundance with high specific radioactivity (2170 Ci/mmol (**66**), is the most commonly used isotope. As a gamma emitter, ^{125}I is easily and efficiently counted without the need for scintillation fluids (**43, 100, 101**). Tritium or ^{14}C labelled ligands (specific activity: 29.2 Ci/mmol and 62.4 mCi/mmol respectively (**102**) have lower specific activities (**67**) and require the use of a liquid scintillant (**65, 67**). For the latter two tracers a greater mass of radio-ligand is required to match the counting efficiency of an iodinated radio-ligand: the result is decreased assay sensitivity. RIA like assays achieve their maximum sensitivity when ^{125}I label systems are employed (**99**).

Ideally the ABs should have similar affinity for both radio-ligand and analyte (**84**). Tritiated ligands, which are often available commercially (**68**), usually bear a closer resemblance to the unlabelled hapten than do iodinated ligands. ^{3}H's longer half life (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 (**66, 103**) mainly because these compounds can be difficult to iodinate without affecting their antigenicity. Tritium based assays tend to be less sensitive than their iodinated counterparts (**67, 80**); although, Weiler et al. (**86**) points out that the difference can be slight.

To minimize steric hinderance by the large iodine atom the hapten can be derivatized with an easily labelled phenolic or imidazole group (**104, 105**). Tyramine or

histamine units that can be separated from the parent molecule by a spacer arm (**106**) are common choices; the subunits may be labelled before or after conjugation to the hapten (**65, 101, 107**). The spacer arm can affect assay performance. If the radio-ligand's spacer arm too closely resembles the immunogen's, the tracer may to be more avidly bound than the analyte, which can reduce assay sensitivity (**108, 109**). Bridge recognition is the chief disadvantage of radio-iodinated tracers (**109**). [^{125}I] based assays that use homologous bridges are frequently less sensitive than their ^{3}H counter parts (**109**). Heterologous linkages that differ in either structure or site of attachment to the hapten can reduce the effect of bridge recognition (**98, 105, 109**). ^{125}I is substituted into the hapten by chemical (eg Chloramine-T) or enzymatic (eg lacto-peroxidase) driven reactions. These reactions oxidise Na^{125}I; cationic ^{125}I is then rapidly incorporated into the phenolic or imidazole residue (**65, 66, 100, 101**). Radio-ligands of high specific activity are essential for sensitive RIAs; the incorporation of one iodine atom per molecule is considered desirable (**105**).

Separation Systems:

With the exception of scintillation proximity assays (**111**), the reliability of RIAs critically depends on the physical separation of the bound and free radio-tracer fractions. Phase separation procedures should be efficient, simple, inexpensive, and should not disturb the equilibrium between the binder and ligand (**66, 112, 113**). Most of the popular separation methods exploit either physicochemical or immunological principles.

The physicochemical methods, which include fractional precipitation by salts or solvents (**84, 100**), are simple, fast, cheap, and reproducible but tend to have high assay blanks (5-20%) (**102, 112**) which can affect assay performance at trace analyte levels (**65, 66**). Such blank effects can be reduced by washing the precipitate (**80**). Adsorption of free tracer by dextran coated charcoal (DCC) is widely used in hapten assays (**100, 114**). DCC methods are convenient, inexpensive, and well suited to large sets of assay tubes (**65**).

The double antibody separation (DAB) technique (**100**), which is probably the most popular of the immunological separation methods, exploits the spatial separation of the ABs antigenic and binding sites. Antibodies raised against the primary host's IgG are used to precipitate the AB-ligand complex. DAB methods are reproducible, non-disruptive (**80, 98, 115, 116**), and usually have low assay blanks (1-3%) (**65, 115**).

Either the primary or secondary ABs can be immobilized on a solid phase (**106**) such as polypropylene tubes (**65, 100, 106**) or particles of latex, sepharose, or sephadex (**65**). Immobilization of the ABs on magnetizable particles can eliminate the need for centrifugation (**92, 102**). Solid phase methods are versatile, have low non-specific adsorption effects (**100, 102**), and have fewer error sources than other methods (**116**). On the debit side, particulate solid phase reagents are expensive, and immobilization may reduce the serum's avidity (**65, 100**).

Because of their non-competitive design, assays that employ labelled ABs usually have the potential to be more sensitive than competitive binding assays, such as RIA, which use labelled analytes (**99, 118**). Immunoradiometric assays (IRMA) use labelled ABs in excess quantities and are non-competitive (**64**). Although the sensitivity of non-competitive assays could theoretically be improved down to a detection limit of a single molecule if a sufficiently active tracer and detection system were available (**99**), their sensitivity is limited in practice by the label's specific activity (**64, 99**). For that reason IRMAs are unlikely to prove more sensitive than conventional RIA methods (**64**) unless a non-isotopic label is used. IRMA is not suited to small haptens since it requires two well separated epitopes per target molecule.

In scintillation proximity assays second ABs which are coated onto micro spheres that contain a fluorophore are used 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.

Enzyme-Immunoassay:

Concern about the health effects of low level radiation and strict regulations governing the use of radio-isotopes stimulated interest in alternative IA labels. Enzyme-immunoassays (EIAs) which were introduced by Engvall and Perlmann (**119**) and van Weeman and Schuurs (**120**) resemble RIA, except that enzyme activity rather than radioactivity is measured. EIAs overcome some of RIA's limitations (**69**) through improved safety, superior versatility (**304**), and longer lived reagents (**100, 121**). The EIA and RIA formats are comparably sensitive (**122**) and cost effective. Moreover, EIAs can be easily adapted for use in the field (**123**).

On the other hand, enzymatic end points are more difficult to determine precisely than gamma emitting labels and they require an extra assay step (**121**). Slight variations in reaction conditions can affect enzyme activity (**76**), although the differences in precision between state of the art EIAs and RIAs are now considered marginal (**100, 122**). Enzyme based assays, particularly homogenous EIAs, may be more susceptible to interferences than RIA (**121**).

In the present review the term EIA is used to describe all IAs that depend on enzyme labelled reagents (**121, 122**) for the signal quantification step. A multiplicity of EIA formats have been developed. The characteristics of the main EIA variants are described in the next section. The application at hand and the nature of the sample matrix usually determine which format is preferable. Most commonly, antibodies or coating antigen are adsorbed on a solid phase to facilitate phase separation (**76, 124**); usually a 96 well plastic micro-titre plate is used for this purpose. The coated plates are stable for 3 to 6 months (**125**). The optical density (OD) of the developed plates are read in micro-titre plate readers which are available from commercial sources. The levels of the various reagents are selected by means of checkerboard titrations (**76, 96**).

Enzyme Tracers: The most popular enzyme labels are horse radish peroxidase, glucose oxidase, alkaline phosphatase, and b-galactosidase (**76, 100, 122, 123, 126**). A variety of reagents including glutaraldehyde and sodium periodate (**123**) are used to conjugate enzyme labels to antibodies. The methods listed in Table 2 can be used to conjugate enzymes to haptens (**76, 122, 123, 125**). Bridge recognition by the induced ABs can be reduced by means of a heterologous assay design or affinity purified serum (**86**).

EIA Formats

EIAs can be divided into two main categories: competitive and non-competitive (immunometric) assays (**124**). These categories can be further sub-divided into heterogenous and homogenous assay formats. Unlike homogenous assays, heterogenous assays require separation of the bound and free phases before the enzymatic end point is determined.

Homogenous EIAs

In competitive homogenous EIA (enzyme-multiplied IA technique (EMIT)) the enzyme is conjugated to the hapten and its activity is modulated by the binder (**122**). EMIT assays are efficient and precise (**100**). Because the mechanism of enzyme inhibition is steric, EMIT assays are most suited to small antigens or haptens (**127**) whose antibody binding sites are close to the conjugated enzyme. EMIT assays are extensively used in clinical chemistry (**127**) and tend to be less sensitive than their heterogenous counterparts (**50, 123**); they are also susceptible to matrix interferences (**127**).

Non-competitive homogenous EIAs (enzyme-immunometric assays) are equivalent to IRMA except that an enzyme tracer replaces the antibodies' isotopic label. They use an excess of labelled antibody and are capable of low detection limits.

Heterogenous EIAs

Competitive heterogenous EIAs are the equivalent of conventional RIAs (**100, 122**) and are the most popular format for residue tests (**50, 52**). They are often loosely referred to as enzyme linked immunosorbent assays (ELISA). The antibody (direct version) or antigen (indirect version) is adsorbed on the solid phase; antigen adsorption helps to conserve antiserum. Immobilized AB versions require the co-incubation of sample and enzyme tracer which can expose the tracer to harmful matrix components (**128**). For the indirect versions a calibrated amount of protein-hapten conjugate is immobilized on the solid phase; the sample and a limited amount of antiserum are then added and incubated. After a phase separation step, the amount of primary antibody bound to the adsorbed hapten is used to indirectly quantify the analyte (**70**). Either the primary or a secondary antibody may be labelled. The use of enzyme labelled DABs, which are available commercially, prevents possibly harmful matrix components from coming in contact with the enzyme label (**70**).

Non-competitive heterogenous (EIA): Although the acronym ELISA is often loosely applied to all solid-phase IAs that use enzyme labelled reagents (**121**), it can also be used more restrictively to describe all non-competitive solid phase heterogenous assays (**123**). ELISAs are commonly used to detect antibodies (**76**) and are excess reagent assays in which the amount of bound enzyme label is directly proportional to the analyte's concentration (**123**). The majority of ELISAs employ enzyme labelled antibodies. If the antibodies are immobilized, the antigen must have multiple antibody binding sites (**121**).

Fluorescent and Chemiluminescent Labels

Fluorescent:

Fluorescent labels, such as fluorescein, rhodamine, and the rare earth chelates have emerged as promising non-isotopic labels (**100, 129, 130**). The labelling techniques are

relatively simple and the tracers have an indefinite shelf life (**129**). The quantification of fluorescent labels rivals gamma counting for rapidity and precision (**129**). Fluoroimmunoassays (FIAs) have been developed in competitive and non-competitive formats. The use of the chelated rare earth fluorophores should help improve the sensitivity of the FIA technique (**130**) by reducing the effects of background signals, which have caused problems in earlier FIAs. It may be possible to extend the sensitivity of non-competitive labelled AB type assays below the limits imposed by radio-isotope labels (**99**) by using high specific activity fluorescent labels.

Chemiluminescence:

Chemiluminescent labels, such as luminol, can be used as tracers in CIA assay systems that in several cases have proven as sensitive as RIA or EIA systems (**131**). The luminol label is oxidised in the presence of H_2O_2 and a catalyst such as microperoxidase (**132**). Wider use of the CIA technique has been impeded by inefficient detectors and the tendency of sample components to interfere with signal detection.

ENVIRONMENTAL APPLICATIONS

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 and assay validation shall be considered in subsequent sections.

Halogenated Aromatic Hydrocarbons (HAHs) and Related Compounds

Several groups of halogenated aromatic compounds have become 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 '70s, researchers at the National Institute of Health Sciences in the USA, recognising the potential of IA as a screening tool, developed IAs for the following HAH pollutants: polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs) (Table 2). This pioneering research overcame some serious problems related to the poor solubility of HAHs and the development of suitable radio-ligands. It successfully demonstrated the ability of IA screening techniques to detect members of this important class of environmental contaminants.

PCDDs

The 75 PCDD congeners, commonly known as "dioxins", have caused more concern than almost any other organic pollutant. 2,3,7,8-T_4CDD, 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 (**19**), there is continued controversy about the nature and magnitude of the "dioxin threat" to human health. Kimbrough (**134**) states that, based on present data and known exposure levels, the PCDDs may not be a serious health hazard for the general population. Recent evidence, however, indicates that PCDDs, PCDFs, and co-planar PCBs not only cause a series of effects at the subcellular level, many of which are manifested clinically in animals and humans (**53, 135**), but are also apparently carcinogenic in humans, where they may act as long term promoters of a variety of cancers (**136**). PCDDs are lipophilic and recalcitrant compounds that tend to bio-accumulate in the food chain. They are formed as by products during the manufacture of chlorophenols and phenoxy herbicides, and during the chlorine assisted removal of lignin from pulp products. PCDDs are also 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 - $2000 per sample).

Albro et al. (37) developed an RIA for PCDDs in an effort to reduce the number of samples that must be analyzed by mass spectrometry.

Because AB - antigen binding is an aqueous phase reaction, hydrophobic analytes such as PCDDs should be solubilized in the assay buffer (137). The solubilization system should render the ligands accessible to the ABs without unduly interfering with the binder - ligand reaction. The non-ionic 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 (15-20%) more than Triton (1-2%) (39). 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 2), and high affinity for the dioxin portion of the radio-ligand (139).

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 only weakly (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 affinity 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 radio-ligand with higher specific activity (Table 3) or a more avid serum (137) would help improve the assay's sensitivity.

Dimethyl sulfoxide (DMSO) also proved useful as a solubilization agent in the RIA for PCDDs (140): a DMSO based assay outperformed Cutscum, Triton, and horse serum solubilization systems at low 2,3,7,8-T_4CDD levels. 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 h 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 (141).

The radio-ligand used in the foregoing versions of the RIA for PCDDs (^{125}I-valeramido-PCDD) had several shortcomings that adversely affected assay performance and reliability. The unlabelled hapten was unstable: it tended to lose iodine during storage and to cyclize, which resulted in variable yields from the labelling reaction. Also the specific activity of the radio-ligand could not be readily increased by an enrichment step. Collier et al. (142) synthesised 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 analogue of the molecule was labelled with cold iodine using a bi-phase reaction that was driven by chloramine-T (143). For unknown reasons the tyramine hapten, 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-TCDD (ChemSyn, Lexana, Texas) provided an alternative radioligand (144) for use in the RIA. 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. The assay used DCC to separate the bound and free phases (Table 3; (144)). The intra-assay precision of the [^{3}H] based assay was apparently better than that of the ^{125}I based assay's (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 - 200 pg of 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. (145) used a novel solid phase RIA to screen hybridoma cultures for the production of anti-2-NH_2-3,7,8-T_3CDD MABs. 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 (146, 147) generated five MABs that were each capable of binding free 2,3,7,8-T_4CDD. 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 MABs differed in their ability to recognize PCDD/PCDF congeners other than 2,3,7,8-T_4CDD; they favoured congeners with intermediate levels of chlorination (**148**). 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. Because the ABs 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 bound. A later version of the EIA for PCDDs used Cutscum as the solubilization agent (**97**); assay performance was optimal at Cutscum levels of 0.125 and 0.25% (v/v).

A sensitive RIA that could be used to detect 2,3,7,8-T_4CDF in commercial preparations of PCBs as well as in environmental samples has also been reported (**38**). PCDFs are present as contaminants in PCB preparations, and are also formed in large quantities when PCBs are burned. The assay had a working range of 20 pg to 4.0 ng of 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 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.

PCBs

PCBs are widespread environmental contaminants that are known to cause a variety of acute and chronic toxic effects in test animals and humans (**90, 151, 152**). 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 (**152**). 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. (**39**) prepared antisera to several PCB derivatives (Table 2) for use in an RIA. The sera had greatest affinity for their corresponding haptens and were fairly specific for their matching isomers. The serum raised against 2-NH_2 -4,5,3',4'-TCBP appeared to recognise the hapten's NH_2 group 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 (43%) than 2,6,2',6'-TCBP (27.3%). Small quantities of detergent were used to solubilize the hydrophobic analyte and radioligand. 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 the 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 to be improved before it could be considered for routine use; that would probably require a more avid serum.

Subsequently, Newsome and Shields (**154**) exploited the tendency of the immune system to recognise -NH_2 groups as Cl atoms by raising an antiserum to 2-NH_2-2',4,4',5,5'-P_5CB (Table 2). The antiserum was expected to be more sensitive to the higher chlorinated PCBs that are common in Aroclor 1254 and 1260. A radioligand with high specific activity (2073 Ci/mmole) 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 antibody-ligand binding than Cutscum. Furthermore, the inclusion of DMSO in the DCC phase separation reagent improved assay sensitivity ten fold, presumably by improving the ligand's access to the charcoal particles. 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 (MN) and Immunosystems Inc. (Maine) recently reported the development of an EIA that is highly sensitive for Aroclor 1248 (**155**).

Johnson et al. (**156, 157**) reported RIAs for 4-acetaminobiphenyl and N,N'-diacetylbenzidine, which are metabolites of the carcinogens 4-aminobiphenyl and benzidine (Table 2). The radioligand was prepared by the coupling of 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. It should be possible to use a similar strategy 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 (**158**) (Table 2). 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. Westinghouse Bio-Analytic Systems developed an indirect EIA for the wood preservative pentachlorophenol (PCP) (**159**). 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.

Herbicides

Phenoxy acid herbicides: The widely used chlorinated phenoxyacetic acid (2,4-D and 2,4,5-T) herbicides 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 (**160**) whose RIA used an antiserum that was raised against the 5-NH_2 derivative of 2,4-D (Table 2). Because the ABs recognized the NH_2 group as a chlorine atom the assay was more sensitive to 2,4,5-T than to 2,4-D (Table 4). A phenolic derivative of 2,4-D was labelled 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 (**161**). In order to improve the specificity of the IAs for 2,4-D, Fleeker (**161**) raised sera to immunogens that were prepared by conjugation of the carrier protein to (a) the acetic acid moiety and (b) the NH_2 group of 2-chloro-4-amino-phenoxyacetic acid (Table 2). The former serum was insensitive to 2,4,5-T, but the latter had comparable affinity 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 could reduce the incidence of false positive results.

Knopp et al. (**162**) generated a low titre (1:50) serum to a 2,4-D hapten that was conjugated through the acetic acid group to the carrier protein (Table 2). 2,4-D was unable to compete for antibody binding sites with an ^{125}I-tyramine derivative of 2,4-D (**162, 163**): probably because the radio-ligand and immunogen were overly similar. A sensitive assay was developed, however, using tritiated 2,4-D (**162**) as radioligand (Table 4). The highly selective serum discriminated against 2,4,5-T (cross reactivity (CR) = 9%). The authors suggested that the serum's titre and avidity could probably be improved through the use of a different spacer group in the immunogen. The acetic acid group was also used Hall et al. (**164**) as a conjugation site in the production of an anti-2,4-D serum that showed little cross reactivity towards 2,4,5-T (11%) and monochloro-phenoxyacetic acid (16%). A novel radio-ligand was prepared by coupling tritiated glycine to 2,4-D via a mixed anhydride reaction. The serum was also used in an EIA for 2,4-D.

<u>Triazines</u>: Dunbar (**165**) and Huber (**166**) concurrently developed a pair of IAs for Atrazine, a popular herbicide that is used for the pre- and post- emergence control of annual weeds in a variety of crops including corn (maize). Dunbar's serum, which was initially described in a patent application (**165**), was raised against a hapten that was conjugated through the 4 position through the 4 position on the aromatic ring to carrier protein (Table 2). A tyrosine methyl ester derivative of the hapten, suitable for labelling with ^{125}I, was synthesised using the mixed anhydride method. For reasons of convenience an indirect EIA format was pursued (**167**). 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 (**166, 300**) version of the atrazine EIA used a serum that was raised against the sulfoxide derivative of ametryn: ametryn is an analog of atrazine (Table 2). Purification of the ABs by immunoaffinity chromatography (IAC) improved the assay's detection limit a hundred fold (Table 4). By immobilization of the ABs 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 was the most reactive (106%) of several triazine herbicides that were detectable by the assay. An attempt by Sharp et al. (**169**) 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. (**61, 170**) are reported to have developed a highly specific anti-cyanazine serum (Table 2) which was used in a sensitive assay. The assay did not cross react with other commercial triazine herbicides but could detect metabolites of cyanazine (**61**).

Huber and Hock (**172**) employed their previously proven techniques to develop an EIA for terbutryn (Table 2). Terbutryn is a post emergence triazine herbicide that is used with several winter cereals; it is also 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 ideally suited to water analysis, was used (Table 4). The improvement was realised without any pre-treatment of the sample (**173**).

More recently Wittmann and Hock (**168**) used an immunogen that contained 35 atrazine substituents per molecule to generate a serum that was used in a very sensitive

assay (Table 2). 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 4 atrazine residues per molecule of BSA was used for an initial immunization period of five 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 was particularly affected (max. CR = 20%). Prof. Hock's group subsequently published an evaluation of 3 versions of the atrazine EIA that were based upon different combinations of 2 sera and 2 tracers (**174**). The sera were raised against two immunogens. The most sensitive assay cross reacted with propazine (195%) and simazine (20%). Wittmann and Hock (**175**) have also developed a sensitive EIA for deethylatrazine and deisopropylatrazine, which are key atrazine metabolites. The immunogen was raised against the 2-aminohexanecarboxyllic acid deethyl- atrazine analogue (Table 2) which was prepared using a CMC coupling reaction. The enzyme labelled hapten was prepared using a CDI/NHS active ester procedure (Table 2). Microtitre 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. The alternation of immunogens with high and low hapten substitution ratios in the immunization protocol once again resulted in a sensitive serum of high titre. The assay was able to efficiently detect both target compounds.

Two versions of an enhanced luminescent IA for the detection of atrazine and simazine have been described (**176, 177**). In one version peroxidase labelled 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 microtitre version of the assay could be developed if a suitable plate reader were available. The reagent concentrations were increased sos as to shorten the incubation time of a version of the assay that is suitable for field use. An outline report has also been made of a FIA for the quantification of

triazine herbicides (178). The tracer was prepared by labelling hapten molecules with fluorophores such as dansyl chloride and fluorescein. A simple, rapid and sensitive tube based 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 (179) (Table 2). 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 microtitre 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 (180).

Schlaeppi et al. (181) developed some highly specific anti-triazine MABs against haptens that were prepared by coupling valeric acid derivatives of atrazine and hydroxyatrazine to carrier protein (Table 2). 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 recognised propazine (CR = 90%) and had reduced cross reactivity to other triazine herbicides and their hydroxylated metabolites. Giersch and Hock (182) conjugated ametryn sulfoxide and dichloroatrazine to BSA in order to raise anti-atrazine MABs (Table 2). Four MABs were selected for detailed characterisation. The MABs raised against ametryn sulphoxide 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 (183, 184) tackled the issue of triazine assay selectivity by preparing a library of atrazine and simazine haptens (Table 2) that could be used to tailor assay specificity and sensitivity to suit analytical needs. 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.

Paraquat: The frequent implication of paraquat 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 (**185**) reported the production of anti-paraquat ABs which were used in a sensitive and selective RIA (Table 4). An optimized 30 minute assay version had a reported sensitivity of 12 pg/100 μL (**186**). Fatori and Hunter (**187**) developed two versions of an RIA for the detection of paraquat in serum and aqueous samples. One version used tritiated paraquat as radio-ligand and was most suited to rapid clinical applications. A more sensitive version, based on an iodinated tracer, was slower (2h) but could detect lower concentrations (Table 4). Niewola and co-workers at Imperial Chemical Industries (ICI) (**188**) developed and systematically optimised an indirect EIA for paraquat (Table 2). Diethylparaquat (40%) (**189**) and monoquat cross reacted in the assay (**189**). 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 set-back prompted a decision to develop anti-paraquat MABs so as to ensure a consistent supply of ABs (**189**). 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%) (**189, 190**). A version of the assay suitable for the analysis of paraquat in soil has been described (**190**). The anti-paraquat MABs were later purified

by affinity chromatography (**191**) in order to remove unwanted mono-valent ABs. Nagao et al.(**192**) used the same hapten as the ICI team to develop anti-paraquat MABs (Table 2) that had much reduced cross reactivity to diethyl paraquat (8%) in a heterologous EIA; although this assay was less sensitive than the ICI group's (Table 4) homologous assay.

Prof. Hammock's team at the University of California (UCLA) (**193 - 195**) also undertook extensive research that led to a PAB based EIA for paraquat. They evaluated four carrier proteins in their assay: conalbumin (CONA) and keyhole lymphocyte albumin (KLH) were the best immunization and coating antigens respectively. A valeric acid linkage system worked best with both antigens (Table 2). Particular care was paid to the structure and concentration of the coating antigen: assay sensitivity is usually enhanced when the ABs do not preferentially bind the tracer or immobilized ligand. The serum and sample were pre-incubated for 24 hours. The assay cross reacted strongly with the methyl propyl analogue of paraquat but showed low recognition for diethylparaquat and other potential interferences.

Other Herbicides: Both FIA and EIA assays have been developed for the detection of the weed grass control agent diclofop-methyl (**196**). The hapten was labelled with fluorescein and enzyme labels by means of mixed anhydride reactions. The AB's inability (Table 2) 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-dichloro-phenoxy)phenoxy moiety.

Clomazone, the active ingredient of the pre-emergence/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 (**197**). An EIA for the detection of clomazone in soil extracts (Table 2) did not cross-react significantly with metolachlor, metribuzin, or trifluralin: three herbicides that are often

used in combination with clomazone (**197**). The FMC Corp. (New Jersey) has also developed an EIA for the measurement of Clomazone in soil (Table 2) (**198**). 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 have used an EIA to screen water samples for the presence of Alachlor. Alachlor is the active ingredient of the widely used Lasso formulation and is also a component of the herbicide mixtures Bronco and Alazin (**199**). Thiolating reagents (Table 2) were used to conjugate alachlor to BSA (immunogen) and sheep IgG (coating Ag) by heterologous linkages. The serum and sample were pre-incubated for one hour. Systematic optimization of the coating antigen and serum levels resulted in a sensitive (Table 4) assay that was acceptably precise over the assay's working range (coefficient of variation (CV) of 4.2% at 0.2 ppb and 18.6% at 8.0 ppb). The antibodies only weakly recognised some other chloroacetanilide herbicides, probably because the immunogen was prepared by linking the carrier protein to the hapten's chloroacetamide group which is common to this class of herbicides. Some thioether analogues of alachlor that are formed as animal metabolites did cross-react in the assay. Those compounds, however, are unlikely to be found in the aquatic environment and should not detract from the assay's usefulness as a screening tool. Researchers at Ciba-Geigy in Switzerland have developed a MAB based EIA for the related herbicide Metolachlor (**200**) which is the active ingredient of the selective herbicide Dual. A distal-carboxylic derivative of metolachlor that was designed to optimise the assay's sensitivity, was used to prepare the immunogen (Table 2). 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, a pre-emergence herbicide that is used with rape, potatoes, and other crops (**201**), the target compound was directly coupled to sulphydryl groups that were introduced into BSA by cleavage of disulphide

bonds with DTT (dithiothreitol) (Table 2). A direct assay format was used in which the PABs were immobilized in microtitre wells. The ABs were highly selective for Metazachlor. Several Metazachlor derivatives and chloroacetamide herbicides cross reacted less than 1%.

Residues of maleic hydrazide, a synthetic plant growth regulator, are of concern in beets, potatoes, onions, and tobacco. Harrison et al. (**202**) described two hybridoma cell lines that produced MAbs with high specificity for maleic hydrazide. 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 was also less tolerant of sample variability (pH, ionic strength, and residual solvent), than a homologous system. Such interferences can be readily controlled in water samples. Later evaluation of the assays using potato extracts (**314**) showed that the heterologous format did not offer a significant advantage mainly because it yielded more variable data.

A du Pont research team developed a PAB based EIA for the detection of chlorosulfuron, the active component of Glean Herbicide (**87**). Sample and serum were pre-incubated for one hour. Chlorosulfuron's main sub-units did not cross-react in the assay. Although two other herbicides that closely resembled chlorosulfuron's bridge and heterocyclic ring structure cross reacted, two related herbicides with different bridge and heterocyclic ring structures had greatly reduced cross-reactivity than chlorosulfuron.

Concern about residues of the selective herbicide molinate in drainage canals and receiving rivers near rice paddies prompted the development of a PAB based EIA (**204**). 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 2); several rabbits were inoculated with each immunogen. This multi-pronged approach increases the likelihood that the optimised 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 2). The samples and standards were pre-incubated with serum for 16 h. Sera that had greater affinity for the coating antigen than the free analyte were discarded. An S-2(p-aminophenyl) ethyl derivative of molinate coupled to CONA was selected as the coating antigen 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 (**164**) for picloram, 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 radio-ligand. None of the related herbicides that were tested could inhibit tracer binding by 50%. These researchers later developed anti-picloram MABs (Table 2) which were used in an effective EIA (**205**). 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.

Aminotriole (MW 84.1) is a non-selective herbicide that is used in fruit orchards and in the preparation of soil for some crops including kale and maize. Jung et al. (**206**) reported efforts to develop an EIA for the detection of this water soluble analyte. A library (Table 2) 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 optimised heterologous assay. The pH of the incubation mixture affected the binding properties of the highly specific ABs. The poor sensitivity of the assay (Table 4) suggests that aminotriole was poorly recognised by the hosts immune system. This study

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

Because the post emergent herbicide imazamethabenz-methyl is hydrolysed by plants to the free acid, Newsome and Collins (**207**) opted to develop ABs to imazamethabenz for use in an indirect EIA. The assay was selective for the target analyte and its methyl ester (CR=627%). Li et al. (**208**) addressed the problem of designing a suitable immunogen for use in the generation of sera against the sulfonamide herbicide bentazon. Bentazon presents a special problem since it contains an ionizable NH group. 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 bentazon derivatives and N alkylated derivatives. The immunogens that were coupled through the aromatic ring yielded ABs that recognised N-alkylated derivatives of bentazon but not the free compound. A wide range of herbicides failed to cross react in the assay.
Riggle (**209**) published an account of an insensitive and poorly selective EIA for the pre-emergence herbicide trifluralin. 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 (**210**). 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.

Insecticides

<u>Chlorinated hydrocarbons</u>: The first IA for an insecticide was designed by Langone and Van Vunakis (**35**) to detect aldrin and its metabolite dieldrin. Aldrin is an organochlorine compound that has broad spectrum activity. The immunogen was prepared from a carboxylated derivative of aldrin (Table 2). The same derivative was coupled to tyramine and labelled with ^{125}I (Table 5) to yield a radio-ligand 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 times and 26 times 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 chlordane - another of the cyclodiene insecticides (**211**). Because 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 (**212**) generated an anti-endosulfan serum against an immunogen that was prepared by using a succinate bridging group to separate the hapten from its carrier protein (Table 2). The preparation of enzyme labelled hapten was complicated by endosulfan's hydrophobicity which caused the peroxidase label to precipitate. The problem was solved by synthesising an amine derivative of endosulfandiol which had reduced hydrophobicity. That derivative was then coupled to the peroxidase enzyme: however only 20 - 30% of the enzyme molecules were actually coupled to the hapten using a periodate coupling reaction. The EIA was more sensitive for endrin (CR = 180%) than for endosulfan; aldrin had much lower cross reactivity (16%) (**213**). Several common degradation products of endosulfan were detectable in the same concentration range as the target molecule.

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 they are unstable in heat and light (**214**). Such considerations prompted the production of ABs (**215**) for use in an RIA for S-bioallethrin (**216**). The radio-ligand 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 used in a subsequent EIA (**217**).

Investigators at Shell Research Ltd. have developed two variants of a pyrethrin EIA (**61, 218, 219**). One assay that was based on antibodies raised against 3-phenoxybenzoic acid (PBA) could detect a broad range of pyrethroids and metabolites that contained the PBA moiety. A second serum that was prepared against dichlorovinyl cyclopropane carboxyllic acid (CYP), was used to detect cypermethrin and permethrin. PBA and CYP are plant metabolites of cypermethrin. 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 was also developed (**61**).

Stanker and colleagues at Lawrence Livermore National Laboratory (**214**) developed 3 anti-pyrethroid MABs against acidified phenothrin (Table 2). The hapten's conjugation site was located are far as possible from the phenoxyphenyl group in an effort to maximise 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.

Benzoylphenylureas: The benzoylphenylurea insecticides are nonvolatile and difficult to analyze unless they are derivatized. Wie and collaborators originally researched the development of IAs for diflubenzuron and BAY SIR 8514 (**222**). Several haptens were designed with the goal of optimizing the immune system's recognition of the analyte's benzamide sub-unit (Table 2) (**222**). A carboxypropyl derivatives of diflubenzuron was used to induce useful sera one of which was used to develop three EIAs for the detection of diflubenzuron, BAY SIR 8514, and some of their analogues (**223**). The analyte was

preincubated with the serum for 16h. Diflubenzuron phenylacetate based coating antigens were superior to the carboxypropyl-diflubenzuron antigens because of overly strong binding of the later by the primary ABs. Of two rabbits that were immunized with carboxypropyl-diflubenzuron, 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. A simplified assay version that used labelled ABs had similar sensitivity to the indirect EIA (Table 5).

In a follow-up study (**224**) the effect of bridge recognition on assay sensitivity was explored. Three approaches were used: homologous sites on the hapten were used to link the immunogen and coating antigen 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 antigen was shown to affect the assay's sensitivity and working range. The structure of the immunogen and coating antigens also influenced assay specificity. The study showed that a library of ABs and coating antigens 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 Prof. Hammock's group to successfully develop IAs for a variety of residues.

Organophosphate Insecticides: Parathion 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. (**225**) developed RIAs for parathion using both [^{14}C]ethylparathion (40 mCi/mmol) and ring labelled [^{3}H]-parathion (300 mCi/mmol) as radio-ligands (Table 2). The PABs were highly selective for parathion: only reduced parathion cross reacted appreciably. A radio-ligand 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 (**226**). Parathion's

nitro group was preserved in 3 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 (**225**) may well be optimal for the induction of anti-parathion ABs. It is also possible that parathion's small size presents special problems.

Hunter et al. (**228, 229**) developed a sensitive EIA for paraoxon, which is the primary oxidation metabolite of parathion. The assay, which was intended as a diagnostic aid, used IAC purified ABs which were pre-incubated with the analyte for one hour. The assay's calibration curve was linear to 10^{-10}M (Table 5); parathion was only weakly (<1 %) recognised (**229**), as were p-nitrophenol (0.04%) and diethyl phosphate (0.7 %) which are hydrolysis products of paraoxon (**230**). Brimfield et al. (**230**) 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 2). The MABs from 2 hybridoma lines were purified by affinity chromatography but yielded lower sensitivity assays than the PABs. Significantly, both sets of MABs, did not recognise p-nitrophenol and diethyl phosphate or similar insecticides. The MABs did however cross react with (p-aminophenyl)paraoxon (127 %), parathion (3.6 %), methyl parathion (3 %) and diethyl phenylphosphonate (4.4 %). Anti-paraoxon sera have also been used in an unusual IA format that is based on competition between acetylcholinesterase (AChE) and ABs for free paraoxon (**231**). 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 studies were prepared by linking paraoxon to carrier protein through the phosphorous moiety, thus preserving the nitrophenol group (Table 2). The sera were used in an RIA in addition to the AChE based EIA. The ABs recognised parathion but did not cross-react with p-nitrophenol or diethylphosphate. The ABs had higher affinity than AChE for paraoxon and could 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 comprehensive 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 (232). 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 in influencing 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 2) / capture Ag combinations chosen yielded sensitive assays that had broad specificity. The specificity 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 specific than the heterologous assays. The specificity patterns of the 3 systems were partially non-overlapping and depended on the immunogen and capture Ag used. Consequently the experimental data was re-evaluated on the basis of double test systems. The best combination could detect 83.3% of the phosphorous diethyl ester compounds tested at an estimated detection limit 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 (233). Enzyme labelled 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 labelled hapten is then added and a substrate disk is pinched into contact with the porus disk. The developed colour is read after five minutes.

Hunter et al. (234) also developed MABs against the warfare agent Soman. The techniques used in this study (Table 2) could be used in the production of ABs against 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. (235) studied the specificity of anti-Soman MABs and PABs. 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 Heldman et al.'s (231) anti-paraoxon serum 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. (236) raised anti-MATP sera in chickens and rabbits; the SOMAN derivative MATP (methyl phosphonic acid, p-aminophenyl 1,2,2-trimethyl-propyldiester) was chosen as a model organophosphorous compound. The antigen was covalently bound to micro-titre wells using Schiff's base. The rabbit ABs, which were purified by IAC, produced the more sensitive assay. This research group also produced anti-MATP MABs (237) which were used in a direct competitive EIA (Table 5). Most of the selected MABs were highly specific for MATP. The MAB based assay was less sensitive, but more reproducible, than the PAB assay. The MABs, which could detect free Soman in the EIA (238), weakly recognised free Sarin but should be able to recognise some non-toxic Soman analogues.

<u>Other Insecticides</u>: DDA (2,2-bis (p-chlorophenyl) acetic acid), the chief urinary metabolite of DDT, is useful as an indicator of DDT exposure in humans. Haas and Guardia (33) raised antibodies to a DDA-protein conjugate (Table 2); the ABs detected free DDA in an a hemagluttination test but did not cross react with DDT. Centeno et al. (32) used DDA anhydride that was linked directly to a carrier protein to induce anti-DDA antibodies in rabbits (Table 2). This antiserum was able to bind the benyl amine salt of the aminocaproic acid derivative of DDA. Neither antiserum was used in an IA.

Banerjee (**239**) developed an EIA for the detection of DDA in urine extracts (Table 2). The serum and analyte were preincubated for one hour. DDT, DDE, and DDD cross reacted less than 5% in the assay.

Aldicarb is cumbersome to analyze by conventional GC and HPLC methods (**240**). 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 pre-incubation of analyte and reagents. A heterologous assay design was effective in the elimination of bridge recognition effects. The highly selective assay had a dose response from 15.6 - 2000 ng of aldicarb (**241**). Aldicarb sulfoxide and aldicarb sulfone, which are important degradation products of aldicarb, did not cross react in the assay.

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 (**242**) for these biological insecticides. IAs are ideal for the analysis of high MW proteinaceous compounds, which 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 - 3000 ng/mL (2 step) and 2 - 200 ng/mL (one step) of endotoxin. The EIA procedure was improved (**243**) by pre-treating the polystyrene cuvettes with gluteraldehyde and using toxin-conjugated enzyme in a direct EIA format. The gluteraldehyde pre-treatment is thought to improve the ABs orientation. The modified assay used less serum and had a working range of 10 - 1000 ng/mL. Cheung and Hammock (**244**) used an indirect EIA with a working range of 15 - 1000 ng/mL to monitor the d-endotoxin of B. thuringiensis israelensis. A non-competitive sandwich EIA has been used to measure B. thuringiensis crystal protein during the manufacturing process (**245**).

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 (**36**) raised ABs to 2-aminobenzimidazole (2-ABZI), a degradation product of benomyl and other carbamate fungicides. 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 (**246**).

Newsome and Shields (**247**) used RIA to determine residues of benomyl on food crops. Because 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, which 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 (**248**) later developed EIAs for benomyl and thiabendazole (TFZ). The coating antigens were synthesised from succinamido derivatives of the haptens (Table 2). The antiserum and analyte were pre-incubated for 15 (benomyl) or 30 (TFZ) minutes. TFZ solubilization was aided by 0.1N HCl. The benomyl EIA had a shallower calibration curve but a lower detection limit than the RIA (Table 6). The benomyl EIA's specificity differed from the RIA's: 2-ABZI did not cross react in the EIA. The anti-TFZ serum cross-reacted slightly with MBC and 2-benzimidazoleurea. Bushway et al. (**249**) used Newsome's serum in a rapid tube based EIA for MBC (Table 6) that is now marketed by IMS, Inc.

Analytical efficiency was improved by a factor of 4.5 (250) when an EIA was used to screen food samples for metalaxyl residues. The immunogen and coating antigen were prepared by means of water soluble CDI and mixed anhydride reactions respectively (Table 2). The sample and antiserum were preincubated for 30 minutes. 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 has also been reported for the detection of triadimefon in foods (251). The coating antigen for this assay was prepared from the derivatized hapten (Table 2) 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 should also be amenable to analysis by IA (252). 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.

Fenpropimorph is a difficult to analyze fungicide that requires enrichment and derivatisation; it is thus an ideal candidate for analysis by IA. Jung et al. (253) used a sensitive variant of the direct competitive heterogenous EIA (254, 255) for the detection of fenpropimorph and its metabolite fenpropimorph acid. The microtitre wells were first coated with affinity purified second antibody. Analyte, enzyme labelled 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 1:200000, which is considerably higher than normal, and suggests that the IDAB format may help to conserve a finite

reagent. The IDAB assay format improves precision by reducing colour 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.

The antibiotic Blasticidin S (BLS) which is used as a fungicide in rice culture is currently analyzed by means of bioassays. Kitagawa et al. (**256**) raised anti-BLS sera in rabbits that were immunized using a BLS-protein conjugate that was prepared by a novel reaction using N-(m-maleimidobenzoyloxy)succinimide (MBS, available from Pierce) as a cross linker (Table 2). The ABs were highly specific for BLS and showed little cross reactivity for other commonly used antibiotics.

Miscellaneous Analytes

IAs have been developed for a variety of other pollutants. An indirect EIA was found useful in the analysis of 2-methylisoborneol (MIB), a metabolite of algae and actinomycetes that is a common cause of off flavours in water (**257**). Camphor, a related compound, was used as a surrogate hapten (Table 2). The antisera were raised in goats and the ABs were isolated by chromatography on protein-G. Camphor-ovalbumin (OVA) was used as the coating antigen. Changes to the coating antigen and enzyme reaction failed to improve the assay's moderate sensitivity (5 μg/mL - 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.

Wie and Hammock (**258**) reported a useful EIA for the determination of the widely used Triton X series of non-ionic detergents. Because these surfactants are non-volatile and unreactive, they should be suited to analysis by IA. The assay readily detected all members of the Triton X series, and had a detection limit of about 1 ng/mL of Triton X100. The assay was about 20-100 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 (**58, 260**) in the aquatic environment. Aherne et al. (**261**) 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 5-10 ppt in pre-concentrated 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 (**171, 262, 263, 265, 266**) could also be adapted for use in the analysis of water and biota. Antibodies and associated assays have been reported for aflatoxins (**310, 311**), ochratoxin A (**128, 267, 309**), trichothecenes, and zearalenone (**54, 269 - 272**) among other mycotoxins (**125**). An innovative EIA has been recently developed for the detection of mercuric ions in water (**274**). The assay is based on a MAB that binds specifically to immobilized mercuric ions. The assay had a working range of 0.5-10 ppb and proved as sensitive as cold-vapour atomic absorption spectroscopy. Two hybridoma clones that could distinguish between BSA-glutathione and BSA-glutathione-$HgCl_2$ were selected. The micro-titre plate wells were coated with BSA-glutathione. Water samples were added to the coated wells and incubated for 30 minutes. 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 mM could limit the assay's use for the analysis of seawater.

PAB OR MAB TECHNOLOGY?

Effective environmental IAs have been developed using both poly- and monoclonal ABs. 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 favoured for future methods. One of the main advantages of hybridoma technology is the ability to select clones that produce ABs of desired specificity and sensitivity. Once selected, these clones can be used to produce a virtually unlimited supply of MABs (**59**). The AB supply factor 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 (**193**) - 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 standardised and reproducible methods and this will foster the wider use of MABs (**193**). Eventually MABs will probably become the key component of approved and standardised IAs for the detection of environmental contaminants (**51, 148**). If, as expected, the cost of producing MABs declines as the technology matures, MABs may well become the clonal type of choice (**44, 275**) especially for commercial assay kits.

<u>Assay specificity and AB type:</u> Among the shortcomings of polyclonal sera are poor selectivity and a variable immune response. Because of 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. The breakdown products of many pesticides are themselves highly toxic. Furthermore, the presence of pesticide degradation products may reveal prior contamination with the parent compound. Thus, sera of broad specificity

are suited to many screening tasks. If necessary, steps can be taken to improve serum specificity. The cross reacting ABs can be removed by affinity chromatography. The hapten can be designed so as to narrow the induced serum's selectivity. The systematic design and evaluation of immunogens and coating antigens 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 (**57**). Serum variability can be a frustrating problem. Sera from different animals can have highly variable specificity, even when raised against the same immunogen; even serial bleeds from the same animal can vary (**96**). Serum variability can be dealt with by using large host animals such as goats or horses or by pooling sera (**77**).

The present report contains several examples of assay specificity problems that were corrected by 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 (**160, 161**) was more sensitive to 2,4,5-T than 2,4-D (19% CR). Serum specificity was reversed by the conjugation of 2,4-D through the acetic acid moiety (**161, 162, 164**). Sera raised against atrazine haptens that were conjugated through the 2-(Cl) position to carrier protein (Table 2; **166, 179**) tended to cross-react with a wide range of triazine herbicides. By coupling atrazine through the ethylamino position, Dunbar et al. (**167**) and later Wittmann and Hock (**168**) were able to narrow the assay's specificity. Anti-atrazine MABs (**181**) that were raised against a similar immunogen to Dunbar's had a comparable specificity pattern. A thorough investigation by Harrison et al. (**184**) confirmed that conjugation position and alkyl substitutions can play an important role in the determination of serum specificity.

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 (**204**), chlordane (**211**), endosulfan (**212**),

metalaxyl (**250**), and iprodione (**252**). MABs, however, do not always have superior selectivity to the equivalent polyclonal sera. That point is illustrated by the anti-paraquat MABs and PABs that were generated by Niewola et al. (**189**) which had similar specificities, with the exception of the MABs higher affinity for diethylparaquat (214% vs. 40%).

Hybridoma technology was used to produce ABs that could bind free Soman (MW = 183) after earlier efforts with sera were fruitless (**235**). A MAB based IA for paraoxon was more selective than its PAB equivalent but did cross-react with parathion and p-aminophenyl paraoxon; the MAB assay also had lower sensitivity (**228, 230**).

Vanderlaan et al.'s (**148**) anti-dioxin MABs were selective for the highly toxic PCDD and PCDF congeners whilst discriminating against the less toxic lower and higher chlorinated congeners. Anti-PCDD sera (**37**) could still have a role because of their ability to provide information on a broader range of homologues. 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 (**42**).

In the past, MABs have tended to have lower affinities (**42**), and thus inferior detection limits than rabbit sera (**44**). Because 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 (**70**). The use of multiple mouse strains and improved clonal selection techniques would favour the selection of high affinity MAB clones. This strategy probably offers the best prospects of generating avid ABs for low molecular analytes - such as glyphosate or aminotriole.

ASSAY SENSITIVITY

Ekins (**315**) defines assay sensitivity, which is primarily related to serum avidity, as the imprecision of measurement of zero dose. It follows from that definition that an assay's detection limit (DL) is the least amount of analyte that can significantly ($P<0.05$) inhibit tracer binding (**83**). 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 the B_0 tubes. Alternatively, the concentration - error relationship can be plotted for a heavily replicated (x10) calibration curve; the zero analyte's error is estimated from this plot by extrapolation of the curve to zero dose (**140, 315**). 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 (x10) calibration curve. The amount of analyte that inhibits AB binding by 50% (I_{50}) is a useful indicator of the assay's responsiveness since it establishes the analyte's concentration in the most precise region of the calibration curve.

A comparison of the sensitivities and detection limits of the reviewed methods (Table 3-6) is complicated by the variety of techniques that have been used to estimate that parameter. The detection limit has been most commonly estimated by multiplying the error associated with the measurement of zero analyte by a factor of 1 (**189**), 2 (**181, 200, 204, 212, 230**), 2.5 (**187**) or 3 (**140, 144, 161, 196, 253**). The DLs of environmental IAs have also been estimated from the amount of analyte that inhibits tracer binding by two times the CV (**250**); multiplication of the mean SD of points within the calibration curve by 3 (**252**); estimation of the concentration required to cause a specified decrease in tracer binding (10% - 20%) (**179, 210, 237, 248**); estimation of the lower limit of the calibration curves's linear range (**204, 223, 224, 234, 300**); estimation of non-overlapping ranges of blank and test replicates (**37, 38**); differentiation from noise level (**36**) ; 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 (**202**); estimation of the smallest quantity that can be analyzed in a single analysis with an error of 5% (**175**). Some researchers prefer that the DL lie within the calibration curve's linear region, even though lower concentrations may be detectable (**223, 224**). In those reports that do not provide an assay DL the tabulated values (Tables 3-6) were estimated by the present author from the published calibration curves (**97, 154, 158, 160, 164, 185, 192, 197, 199, 206, 214, 216, 239, 242 - 244, 247, 251**). Many other reports provide a DL but do not describe its derivation (**35, 39, 52, 146, 156, 157, 159, 162, 166 - 168, 172, 173, 182, 198, 205, 211, 225, 231, 233, 236, 240, 256, 302**).

The lower limit of the calibration curve's linear range has also been used to estimate the assay's practical quantitation limit (PQL; **204**). The PQL has also been gauged by multiplying the experimental error associated with the zero analyte by a factor of 10 (**248, 252**). When the calibration curve is prepared in matrix the DL is equivalent to the assay's quantitation limit. Assay performance characteristics often differ between buffer and matrix systems (**83**), and should be determined for each matrix type.

HAHs (summarized in Table 3): The sensitivity of the RIAs for 4-acetamidobiphenyl and N,N'- diacetylbenzidine (DLs < 10 pg) may have resulted from the use of succinamido spacer arms in the immunogens. Newsome and Shield's (**154**) RIA for PCBs also performed well at low analyte levels 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. Because levels in Canadian waters are usually in the region of a few to several μg/L (**117**), 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 (**117**). 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. 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. The performance of the IAs for PCDDs at trace levels will crucially influence their usefulness for screening samples from the aquatic environment. Preliminary data using the ^{3}H- based RIA indicate that the MAB based assay's calibration curve is shifted to the left of the PAB's (I_{50} of 210 vs 350 pg).

Herbicides (summarized in Table 4): Although the performance of Rinder and Fleeker's RIA for 2,4-D and 2,4,5-T (**160**) varied with each batch of tracer it was apparently more sensitive than the corresponding EIA (**161**); 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. (**163**) whose PAB based RIA had a detection limit of 100 pg. Several of the triazine IAs also have excellent sensitivity. Huber's (**166**) use of ABs that were immobilized on polystyrene spheres facilitated a low DL by permitting the analysis of large sample portions (20 mL). Two recent conventional EIAs from the same laboratory had DLs of 20 ppt and 1 ppt of atrazine (**168, 174**). The excellent sensitivity of the latter (**168**) EIAs for atrazine and propazine (Table 4) was partly a result of the choice of enzyme tracer. The tracer was prepared by linking peroxidase to atrazine by 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's innovative immunization protocol was also used to produce a sensitive serum for deethylatrazine and deisopropylatrazine (**175**) which was combined with an efficient peroxidase tracer to produce a highly sensitive assay. The sensitivity of IMS's EIA for atrazine was dramatically improved when the slightly less convenient microtitre-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 (**176**).

Of two MAB based assays for paraquat, one was reasonably sensitive but lacked selectivity (**189, 301**), and the other was highly selective but insensitive (**192**). A MAB based EIA for picloram was more sensitive than the corresponding PAB based EIA (x5) and RIA (x50) (**205**), mainly because its calibration curve was steeper and shifted to the left. Although, the RIA's sensitivity could probably be improved if a radio-ligand of higher specific activity were used. The comparable sensitivities of the paraquat RIAs and the better of the EIAs suggests that neither tracer type is inherently superior.

Several of the IAs for small herbicide molecules such as maleic hydrazide and molinate required a pre-concentration step for low level analyte levels. The mid-curve response of the molinate assay was improved by a factor of 4-5 when the immobilized DAB assay format (IDAB) was used (**52**). This format could probably be used to good effect with other analytes. The sensitivity of the EIA for bentazon (**208**) was improved by derivatisation of the bentazon residues: N-methyl and N-ethyl derivatives were most effective.

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 a factor of x16 and x10 respectively. The EIAs for diflubenzuron (**223**) were as sensitive as conventional HPLC and GLC - electron capture detector (ECD) methods. The MAB based assays for paraoxon and MATP were less sensitive than their PAB equivalents. Although in the case of MATP the MAB's superior specificity probably made up for the loss of sensitivity.

Fungicides (summarized in Table 6): The DL of the benomyl EIA was lower than the corresponding RIA's despite the EIA's shallower calibration curve. The exceptional sensitivity of the IDAB based IA for fenpropimorph may result from the improved accessibility of the AB's binding sites. All the bound ABs are oriented correctly for

effective coupling with hapten molecules. The IDAB format also probably causes less denaturation of the ABs compared to direct coating methods. Because the second ABs are used in large excess all of the primary ABs added to each well are bound to the solid phase which helps to reduce inter well variability (**316**).

Criteria for Sensitive Assays: Assays with I_{50} responses near 1 ng/mL in the final assay buffer have been categorized (**51**) as highly sensitive; an assay with an I_{50} in the 40 - 100 ng/mL range would be considered to have low sensitivity. By these criteria several of the assays listed in Table 3 are sensitive (**37** (triton version); **38, 97** (0.25% cutscum version)) **144, 154, 156, 157**; several are of intermediate sensitivity (**37** (GC5 serum, cutscum version), **39, 97, 147**), and one has low sensitivity (**159**). The assays described in Tables 4- 6 could be similarly classified. One must be aware, however, that assays, such as Hunter and Lenz's IA for paraoxon (**228**) (Table 5) which had a high I_{50} value but a low detection limit (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 detection limit it may be necessary to pre-concentrate the sample, prepare a detailed calibration curve, or mathematically extend the curve's linear range (**204**). A simple pre-concentration 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 can 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 assay sensitivity is primarily related to AB affinity, fine tuning of the assay format and mechanics can help improve many assays. For instance, prolonging the pre-incubation of ABs and analyte may help improve the sensitivity of some assays. The polystyrene sphere version of the triazine IAs (**173**) and the success of the IDAB format (**253**) shows that innovative assay design can improve assay sensitivity. EIA detection limits can also be lowered through the use of signal amplification systems such as biotin-avidin auxiliary labels (**266**). In essence, the biotin-avidin system (**76, 126, 312**) increases the number of enzyme molecules bound to each antigen - IgG complex, this allows a reduction in the amount of primary B used in the assay, so lowering the detection limit.

SAMPLE PREPARATION

The exceptional sensitivity and specificity of the IA technique often permits a reduction in sample preparation compared to conventional methods (**43, 44**). In the case of water samples and physiological fluids sample preparation can often be eliminated. For many matrices, however, there is a point beyond which there is a trade-off between reduced enrichment and sensitivity. IAs, as aqueous phase tests, are generally easier to apply to hydrophillic 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. Non-polar analytes can usually be solubilized with the aid of non-ionic 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 (**42**). The following polar solvents have been used to aid the solubilization of analytes in environmental IAs: DMSO (**141, 154, 208, 225, 252, 303**), acetonitrile (ACN) (**204, 208, 214, 224, 223, 302**), dimethylformamide (DMF) (**209**); methanol (**158, 208, 211, 238, 250, 251, 302**), ethanol (**225, 238, 239, 257**), and propylene glycol (**204, 302**), dioxane (**232**). Solubilization agents should be carefully evaluated for each assay system because of the unique nature of Ab-Ag reactions. For example, a variety of AB

and coating Ag systems that were evaluated for the bentazon IA were observed to have strikingly different tolerances for ACN (10-50%) (**208**).

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 detection limit 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 (**49**). On the other hand, the high cost of analysis for many lipophilic compounds ($1500 - $2000 for PCDDs) is a strong stimulus for the development of screening methods. The amount of sample preparation could probably be reduced for analytes that do not have to be detected at trace levels. Even for analytes, such as dioxins, that must be extensively enriched (**141**), 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 minimise these 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 (**37, 137**) by RIA. 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 (**38**) to prepare Aroclor, liver, and adipose samples that were

contaminated with PCDFs for analysis by RIA. 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 (**97**): the samples were chromatographed on activated carbon and then passed through a combination column that contained acid silica and $AgNO_3$ treated silica. 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 (**141**). 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 by simply increasing the sample size. Higher than optimal increases in the sample size narrowed the assay's working range, adversely affected the dose response, and raised the detection limit. A more rigorous clean-up may help to reduce the assay's quantitation limit. 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 (**154**) passed solvent extracts of PCBs from blood and milk through a column of neutral alumina prior to analysis by RIA. 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 purifying on alumina before analysis by EIA (**214**). The assay's response was linear from 50-500 ppb in the presence of extract. Clean-up by SPE on C_{18} cartridges was

required before extracts of wheat or barley could be screened for imazamethabenz content by EIA (207).

Wie and Hammock (223) did not observe matrix interferences when using EIA to analyze diflubenzuron and BAY SIR 8514 in stagnant or WHO synthetic water. When whole milk was analyzed, however, it was necessary (224) to extract the samples with EtAc and use a multi-step clean-up. Calibration curves prepared in the final extract indicated that the extract was interference free.

IA has been used to detect the contaminants listed in Table 7 in crude liquid matrices. IAs can frequently be used to detect analytes in solid matrices after a simple extraction with solvent or acid and re-suspension of the extract in buffer (46). This point is evidenced by the contaminants listed in Table 8 which were analyzed by IA in crude extracts of a variety of matrices. Goh et al. (277) studied the effects of the extraction solvent on the tube version of IMS's EIA for atrazine. In field applications the residual solvent should be diluted to at least the maximum tolerable level; in laboratory applications the solvent can be easily removed. The ability of four solvents to extract atrazine from soil depended on the concentration of atrazine (278). 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 (184). The pH and ionic environment of samples and standards should be similar, which may necessitate adjustments to the assay and diluent buffers (195, 208), otherwise curve shifting effects can occur. Concentrated PBS (10x) can be used to equalize ionic effects in water (159, 204) and juice samples (249). IMS's EIA for triazines has been reported to be resistant to interferences caused by humic substances (279).

Residual interferences can be revealed by the preparation of an assay calibration curve in the presence of the matrix (43). 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 safe guard against residual matrix interferences (**43**). IAC techniques can be used to conveniently prepare matrix blanks (**266**). In most cases slight matrix induced biases should not limit an assay's usefulness.

Solid phase extraction, 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 (**302**). Should a solvent exchange be necessary a small volume of keeper solvent, such as propylene glycol, can prevent losses of volatile analytes (**52**). 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 (**160, 161**); molinate (**302**); PCP (**159**); B. thuringiensis israelensis d-endotoxin (**244**), bentazon (**208**), benzo-a-pyrene (**158**), s-triazines (**279**), and cyanazine (**61**).

Recently, Stocklein et al. (**280**) 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 dependant 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 a solvent change. Anti-progesterone ABs (**281**) have also been observed to retain their activity in hexane although calibration curves were shifted to the right and the reaction rate was slowed somewhat. ABs are thought to retain their activity in solvent because of a residual shell of surrounding water. These observations open the way for bi-phase assay systems that may be applicable to lipophilic compounds.

VALIDATION AND ROUTINE APPLICATIONS

One of the most informative way to validate an IA's performance is to make a critical comparison of its performance with that of an independent control method (**44, 50, 52**). Samples from a variety of locations should be used in any validation exercise because of the inherent variability of environmental matrices. The samples can be sub-divided and fortified in order to test for additivity of the dose response (**97**). The key assay performance and reliability parameters such as sensitivity and precision need to be established for each intended matrix. There is a need for a generally acceptable protocol for the validation of assays (**44, 52**), so that assay developers and end users can have equal confidence. A recent interlaboratory comparison of atrazine EIAs that was held in Germany (**282**) was a step in the right direction. In the USA several federal government agencies (**56**) as well as the Association of Official Analytical Chemists (AOAC) and the International Union of Pure and Applied Chemistry (IUPAC) are reported to be developing guidelines for the evaluation of EIA kits. It has been suggested that screening techniques should not have to meet the same rigid requirements of quantitative methods (**62**). Kaufman and Clower (**56**) recently published an informative account of the review and approval process that has been proposed by the Food Safety and Inspection Service of the US Department of Agriculture. The cornerstone of the proposed approval process will be successful assay performance in a supervised collaborative study. EPA (**56**) are reported to be correctly placing the onus for documentation and optimisation of an assay on the developer and require that adequate pools of key reagents such as ABs be available before an assay can be approved. A method's final acceptance will depend on its performance in a collaborative study.

<u>HAHs and related compounds</u>: Johnson et al. (**156, 157**) validated their RIAs for the detection of biphenyl compounds by demonstrating that the assay calibration curves were parallel to curves prepared in fortified urine samples ($R \geq 0.99$). Newsome and Shields (**154**) compared the ability of GLC and RIA to recover PCBs from fortified milk (20-80 ppb) and human blood (2-16 ppb). 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's EIA for PCP was validated 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 (**159**).

Albro et al. (**37**) assessed the ability of RIA to detect PCDDs in liver and adipose tissue from PCDD contaminated monkeys. There was good agreement between the RIA and the GC-MS and GC-EC techniques. Afghan et al. (**307**) subsequently used RIA and GC-MS to detect 2,3,7,8-TCDD in a variety of fish extracts. 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 was also undertaken (**137**). Each sample (60 mg/tube) was independently analyzed by GC-MS and RIA (3X). The number of false positive samples (5.9%) and the greater than 50% false negative results at the lowest spike level 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 was also evaluated using fortified Lake Trout extracts (**141**). 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 ppb - 1750 ppb of the most toxic PCDDs/Fs (**97**). The enriched extracts were sub-divided 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 (**38**); GC-MS and liquid scintillation techniques were used as control methods. The EIA for benzo-a-pyrene underestimated BP metabolite levels by about 70% (**158**) when a BP reference curve was employed.

Herbicide Assays: RIA was used to accurately recover 250 - 2500 ppb spikes of picloram from river water and urine (**164**). The inter- and intra-assay precision of MAB and PAB versions of a picloram EIA were assessed using calibration standards and fortified samples (**205**). The mean assay precision was excellent for the calibrated standards (intra- < 7%; inter- < 16%). When fortified plant extracts were analyzed the intra-assay variability of the MAB assay (CV% = 10-29) 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 interferences a dose response was established for each matrix. The EIA for chlorosulfuron was also prone to matrix effects (**87**), although it was still possible to establish a dose response for soil extracts. The chlorosulfuron assay's PQL compared favourably with both bioassay and HPLC methods. Schwalbe et al. (**196**) 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 GC and liquid scintillation control procedures.

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 (**181**); the overall variation in recoveries was from 46% - 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 (**179**) had good precision over its working range when used to analyze a variety of water (CV 4.1-23.8) and soil (CV: 6.8% - 20.3%) samples. 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. IMS's atrazine kit has also been validated using food samples (**323**). The EIA for deethyl- and deisopropylatrazine had an interassay CV of 4.5% when used to analyze deethylatrazine controls on 10 different days (**175**). EIA analysis of spiked tap water and environmental was accurate which indicated an absence of serious matrix effects.

A total of 13 laboratories participated in an interlaboratory validation of EIAs for the detection of s-triazines in water samples (**282**). 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), Prof. Hock's Lab., 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 3 best assay systems (REs-i-Quant, Prof. Hock's, Envirogard) each of the IAs correctly identified the negative samples. Test kits that employed broad specificity ABs (Res-i-Quant, Envirogard) yielded higher than normal atrazine levels. The study's organizers made several 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 6 calibration points should be used; 5. if the matrix is unknown a fortification experiment should be undertaken; 6. 4 parallel measurements should be made per sample to guard against erratic results.

Goh et al. (**278**) reported that standards processed using the IMS tube based EIA can give variable results. Analysis of variance (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 (**278**) despite a significant bias on the part of the EIA data that was mostly caused by dilution artifacts. Thurman et al. (**279**) 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. Both methods were comparable ($R \geq 0.91$) over the range 0.2-3 ppb. The correlation was maintained when the samples were enriched by SPE; the data was corrected for cross-reactivity factors. No false negatives were recorded at a DL of 0.2 ppb.

Sharp et al. (**169**) assessed the performance of their EIA for triazines by comparing the ability of the IA and a conventional GLC/nitrogen phosphorous 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 (**172**) found no difference in the performance of the EIA for terbutryn when it was used to analyze filtered river water and tap water. Wust et al.'s (**174**) EIA 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 Harrison et al.'s EIA for atrazine (**184**). Shell Research's EIA for cyanazine (**61**) was validated using a variety of atrazine and cyanazine contaminated samples. 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 (**204**) by the preparation of calibration curves in creek and rice field water. 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 (**50, 302**) method. The IA and GC methods gave comparable recoveries of molinate (1 ppb - 1 ppm) from extracted tap water, 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 detection limit. 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 3x).

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 (**283**). 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. An 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 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 (**314**). 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 (**161**) validated two EIAs for 2,4-D using groundwater samples that had been fortified with [^{14}C]-labelled 2,4-D. The EIA data compared well with the scintillation control method (r = 0.99). Knopp et al. (**162**) used fortified human serum (5-250 ppb) to evaluate an RIA for 2,4-D; analyte recoveries were in the 94-104% range and the inter-assay CV% was a low 5.3. Hall et al.'s RIA for 2,4-D was (**164**) validated using fortified river water and urine (250 and 2500 ppb). The analyte recoveries were

excellent and the intra-assay CV was ≤ 9%. Similar results were obtained for the same groups 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 (**197**). A correlation coefficient of 0.98 was obtained when Dargar et al.'s EIA for clomazone (**198**) was compared with a GLC method. The clomazone EIA was found to agree with the results of leaf damage bio-assay 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 - 200 ppb) from barley and wheat (**207**) over the entire spiking range. A mean recovery of 89.8 % was obtained for four barley samples that were spiked with 12.5 ppb.

A set of two hundred and eight samples from rivers and water treatment plants, some spiked as positive controls, were used to assess the EIA for alachlor (**199**). There was good agreement between the EIA and the confirming GC-MS technique (r = 0.84), although the EIA was less accurate and precise (CV%: 10 -40). The EIA data was re-interpreted 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 EIAs for metolachlor (**200**) were validated in a recovery study that used soils fortified with 20 and 50 ppb of analyte. The mean recoveries for the direct and indirect assays were 98 and 89 % respectively; the interassay CV% were 14.5 % and 14% 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 - 100 ppb) and then analyzing the samples by EIA and GC. The samples were pre-concentrated by SPE using C_8 cartridges. Analyte recoveries ranged from 99% - 118% (S.Dev. = 1.8 - 13.4) and there was excellent agreement between the two methods (R^2 = 0.95) (**208**).

The various IAs for paraquat have been validated using spiked serum (r=0.95) (**187**) spiked soils (r=0.97) (**190, 301**), serum (**187**), plasma (r=0.998) (**185, 308**) milk, potato and ground beef (**195**). Good agreement was established in a comparison of an EIA and RIA for paraquat (**188**) (r = 0.96; n=41). Van Emon et al. (**194, 195**) 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 (**194**); moreover the EIA afforded lower DLs.

Insecticide Assays: 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 - 897 ppm) (**211**). 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 non-spiked urine samples indicated close agreement between an EIA for DDA and reference GC and colorimetric methods (**239**). 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 (**224**). The permethrin EIA (**214**) was validated by demonstration of an approximately parallel relationship between the control curve and a curve that was prepared in meat extract. There was good correlation between the observed and expected levels of permethrin for analyte levels greater than 50 ppb. The EIA for aldicarb (**240**) 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. When used to analyze spiked blood an RIA for paraoxon (**299**) showed good precision (9.5-12%) over the range 0.2-3.2 ng. The ELISA for B. thuringiensis israelensis endotoxin correlated well with a bio-assay control method when used to analyze seven commercial formulations of the insecticide (**243**).

Fungicide Assays: The FIA's estimates of 2-ABZI in spiked water samples were within 5% accuracy in a small scale experiment (**36**). 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 (**247, 249**), metalaxyl (**250**), triadimefon (**251**), iprodione (**252**). 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 (**248**). A GLC method and EIA (**253**) gave comparable estimates of the fungicide fenpropimorph in spiked tap water and soil percolation water. 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.

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.

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. Since IAs do not depend on volatility, thermal stability, or the presence of chromophores (**44**) they are well suited to compounds that are difficult to analyze by chromatographic methods (**43, 44, 58, 62**). Many analytes that usually require derivatisation 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 (**42, 62**). IAs are typically most sensitive towards large molecules that have several polar functionalities. Although ABs can be raised against haptens that are as small as 150 Daltons (**58**), assay quality tends to be poorer for small molecules owing to the preponderance of low affinity and low specificity ABs (**49**).

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. Hydrophillic 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 should 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 (**43, 51**). 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 (**100, 284**) of individual assay steps using liquid handling work stations (**76**), automated micro-titre plate washers, and semi-automated plate readers or radiation counters. Completely automated IA systems are now available for clinical applications (**285 - 287**) some of which can process over a hundred samples per hour (**286, 287**). Because IAs can be used to screen large sample sets at realistic costs (**42, 50, 51, 55, 77**), they should facilitate large scale surveys and monitoring programmes that are currently unrealistic. Analyses that require the detection of multiple analytes in a few samples, however, are best undertaken by conventional means.

IAs 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 (**305**). 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. 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.

Factors Affecting Implementation

IA's have yet to make a significant impact in routine environmental laboratories. In a submission to the Congress of the US, Office of Technology Assessment, Mumma and Hunter (**62**) considered the constraints and opportunities of IAs in light of regulatory and legislative influences. Regulatory agencies were criticised 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 an environmental regulatory agency is a crucial pre-requisite for its wide-spread 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 Dept. of Agriculture, which has in the past supported the development and testing of IAs (**62, 283**), in a pioneering move, has initiated a program to monitor rice paddy water for molinate and thiobencarb by ELISA (**50**). 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 a lead in the production of ABs to their products. Currently, about 50% of Pesticide Manufacturers are reported to have healthy IA projects (**31**). 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 (**52**), 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 understand the attitude of a leading agrochemical corporation that refused to make small quantities of a serum to one of their pesticides available to a government laboratory for research purposes. In Europe the possibility of supplying ABs and immunoaffinity columns through a centralised bureau is being studied (**288**). Once ABs become more readily available, individual users will be free to develop 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 (**49**).

Several companies now market IA kits for environmental and food contaminants (**31, 51**). ImmunoSystems Inc. of Scarborough in Maine were 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. IMS also plan the release of EIAs for picloram, PCB, and PCP in the near future (Harrison, personal communication). More than any other company IMS has pioneered the development and use of commercial IA kits for environmental applications. Millipore now distribute some of the IMS assay systems as their Enviro Gard line of screening tests. The kits are available in the tube format for the following analytes: triazines, cyclodienes, carbofuran, 2,4-D, aldicarb and in the plate format for triazines, alachlor, 2,4-D, aldicarb, and benomyl. Bio-Nebraska market a kit version of thier IA for the detection of mercury (**110**). 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 (**289**) eliminates tube to tubes variation due to uneven AB coating. Assays are available for triazines, aldicarb, and alachlor. Granite Division of Environmental Diagnostics Inc. sells rapid format IA kits for paraquat but reports minimal sales thus far (**290**). Westinghouse BioAnalytic, after having developed and distributed EIAs for PCP and Atrazine later withdrew from the marketplace.

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.

Future Growth

IAs low costs 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 cleanup they could be used to screen groups of humans following accidental or occupational exposure to harmful organic contaminants (**42, 44, 158, 240**). EIA has been successfully used to monitor agricultural workers for exposure to paraquat in a pilot study (**194**). 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.

RELATED TECHNOLOGIES

ImmunoAffinity Chromatography (IAC)

The selective AB-Ag reaction can be used to extract hapten molecules from complex solutions. Antibodies have been used in a novel extraction system to actively extract paraquat from macerated glass fibre filters (**193, 194**). 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; the analyte is subsequently desorbed and eluted. A variety of pre-activated support materials are commercially available. The activated columns are stable (**291**) and can usually be reused many times. For high pressure IAC (HPIAC) techniques the ABs are immobilized on rigid supports that can withstand up to 1000 psi (**133**). 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 (**292**). Hamers and Paulussen (**293**) developed an automated column switching system to link an IAC column with a C_{18} HPLC column for the isolation of nortestosterone from biological samples. Several hundred samples could be analyzed on each IAC column. Researchers at Monsanto Agricultural Company used IAC for the isolation of cytokinins (**294**). The column's capacity was increased by the use of purified IgG, and the method had a dynamic capture efficiency of 100%. A MAB based IAC system was used to enrich chloramphenicol from swine muscle tissue (**295**). Co-extracted molecules did not interfere with the separation, and 100% of the analyte was recovered. IAC has been used to isolate many other analytes such as aflatoxin Mi from milk (**296**); chloramphenicol from milk (1L) and eggs (**297**,), and nortestosterone from defatted meat (**298**) samples. 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.

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 (**306**). Similar probes could be used in non-clinical applications such as the monitoring of effluents or receiving waters (**306**). Bright et al. (**138**) described an immunosensor that consisted of fluorescent AB fragments that were immobilized on the end of a fibre optic probe. When analyte bound to the ABs, a 3-5 fold increase in fluorescence occurred. The sensor could be regenerated more than 50 times and was evaluated using HSA as analyte.

In an alternative format (**149, 150**) unlabelled primary ABs are immobilized on the probe; after contact with the analyte the probe is exposed to fluorescein labelled second AB. The analyte level is proportional to the intensity of a fluorescent signal which 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 (**273**) have 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. The innovative probe consisted of a sensor cell that contained capture ABs which were 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 that 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 p mol of 2,4-dinitrophenol-lysine /200 μl in continuous flow conditions (**153**); it

should be readily adaptable for use with other analytes in water samples. In research that opens the way for the development of multi-channel immunosensors, Hlady et al. (**316**) 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 biosensor probes, such as denaturation and irreversible biding of competing compounds; these must be successfully addressed if immunosensor probes are to become widely used (**75**).

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 **203**). Two types of labels have been explored: enzyme labels that catalyze the production of electroactive product and labels that are themselves electroactive. Several promising assays for clinically important analytes such as digoxin (dl: 50 pg/mL) have been developed. Electrochemical IAs are free from many matrix interference problems and afford detection limits in the low pg/mL range with samples that are as small as 20 μL. The adaptation of flow injection analytical techniques for use with biochemical reagents such as ABs and enzymes is dependent on adequate contact time between the reagent and analyte molecules. This is achieved by the use of stopped flow techniques (**220**). A recently published mini-review has surveyed the FIIA methods proposed so far (**220**). The first FIIA method for the detection of a pesticide in water (**221**) has recently been reported. Anti-atrazine serum was immobilized on immunoaffinity membranes. The membrane is automatically changed after each assay. A "stop and go" sequence was used to pump the individual 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 - 0.3 ng/mL and the method had a detection limit of 0.02 ng/mL. The FIIA's precision was lower than the corresponding EIA, which was thought to have several causes: the nature of the FIIA reaction, the use of non-equilibrium conditions, and the immobilization technique.

Multi-residue IAs: Theoretical and practical considerations restrict the use of current IAs to single or dual analytes (**227**). Multi-analyte IAs would find ready application in clinical and environmental laboratories (**118, 259**). It appears that assay formats based on labelled ABs offer the best possibility for the detection of multiple analytes in small volume samples. Ekins and co-workers (**118, 259, 268**) in a recent series of reports, have described the principles underlying a multi-analyte microspot IA technique. The proposed technique uses high activity flourorescent labels which are about the most sensitive tracer systems available. The ratio of fluorescent signals emitted by occupied and free ABs that are located in spatially separated AB microspots is measured; the AB microspots collectively form an array on the surface of a micro-titre well or of a probe. The authors estimate that the technique, which is in its infancy, could detect as many as 10^6 different analytes in a 100 μL sample. In practice, an array size appropriate to the task at hand would be used. Each micro-spot could be directed against a different analyte. When a small number of "capture" ABs are exposed to the sample, a fractional occupancy occurs that is directly proportional to analyte concentration (**259**) and is independent of the quantity of capture ABs. A labelled second AB can then be used to detect either bound antigen or unoccupied ABs (**118**). Both sets of ABs can be labelled with rare earth fluorophores (**130**) with distinct emission patterns (**99**). The fluorophores would be quantified using time resolved fluorometric detectors (**100**). 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 non-competitive micro-spot array assays should have a sensitivity of 4×10^{-15} M/L, at least the equal of conventional RIA like assays which have a theoretical maximum sensitivity of about 10^{-14}M/L regardless of the tracer system (**99, 118, 259**). The basic principles underlying multi-analyte microspot IAs have been verified using a laser scanning confocal microscope (**118**). This novel assay format has the potential to revolutionise environmental IAs by facilitating the detection of multiple analytes in a single test whilst improving the sensitivity of many competitive IAs whose performance usually falls short of the maximum achievable. Multi-analyte microspot IAs could well be the way of the future.

CONCLUSION

Environmental IAs have much promise because of their sensitivity, low cost, rapidity, ease of use, and ability to process many samples at the same time. The paucity of full scale and rigorous validation studies has impeded the more widespread use of IAs in routine analytical laboratories. Some of the recently published validation studies should increase confidence in the ability of IAs to deal with real world samples. The high initial cost of assay development and the previously poor availability of ABs to priority pollutants have been further impediments. As the foregoing problems are overcome, environmental IAs will become as valued by residue analysts, as their counterparts are by clinical chemists.

APPENDIX

APPENDIX 1

IAs for glyphosate and diquat

This appendix considers the suitability of the herbicides diquat(1) and glyphosate(2) as target molecules for analysis by IA. There is an strong demand for a screening method for glyphosate, which is a non-selective post-emergence herbicide that is effective on deep rooted perennial species, and annual and biennial species of grasses, sedges and broad-leaved weeds. Glyphosate is water soluble and its residues are determined by HPLC methods. Diquat is a contact herbicide that is rapidly absorbed by green plant tissues which are subsequently killed on exposure to light. It is often used in combination with paraquat to control grasses. There is a greater need for a screening method for glyphosate than for diquat.

Because of their molecular sizes (glyphosate: 169.1; diquat: 184.2) both molecules m2ust be conjugated to a carrier protein before being used to induce ABs. The small size of both molecules would tend to make the induction of high affinity ABs problematical. Sensitive assays have been developed for paraquat which is related to diquat and to parathion and paraoxon which are similar to glyphosate.

A possible conjugation route for diquat would be to aminate the aromatic structure in the para- position by nucleophilic substitution followed by conjugation to carrier protein via a diazo reaction. A similar strategy has been used to develop anti-parathion and anti-clomazone sera. Alternatively, a spacer chain can be introduced between the hapten and the carrier protein using a mixed anhydride reaction. A similar strategy has been used to synthesise anti-dioxin haptens.

Glyphosate's small size and lack of distinctive molecular structures may also make the induction of avid sera difficult. In the absence of empirical evidence, however,

it is difficult to be conclusive on this point. In the case of the similar compound Soman, PABs were found to weakly recognize Soman but to cross-react strongly with various Soman analogs. Glyphosate could be coupled to p-aminophenol to form an ester which could then be conjugated to carrier protein by a diazotization reaction. This immunogen could then be used to induce the formation of PABs. If the sera did not have high enough affinity for glyphosate hybridoma technology could be employed to isolate the small number of clones that selectively and avidly recognise the immunogen's glyphosate moiety. As has been explained in the body of the review this is a more costly exercise. An alternative route for the synthesis of a glyphosate immunogen would be to couple the hapten to carrier protein via the carboxyl group using an NHS active ester reaction.

Once suitable sera are developed they could be readily used in an indirect EIA format using an enzyme labelled second AB as the tracer. It should be possible to analyze both glyphosate and diquat in water samples without the need to pre-treat the samples.

APPENDIX II

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
CR: cross reactivity
CDI: carbodiimide
CMC: 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-4-toluene sulfonate
CONA: conalbumin
CV: coefficient of variation
CYP: dichlorovinyl cyclopropane carboxyllic 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
DMSO: dimethyl sulfoxide
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
GPC: gel permeation chromatography
HAH: halogenated aromatic hydrocarbon
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
IAs: antibodies immobilized on plastic spheres
ICI: Imperial Chemical Industries
IDAB: immobilized DAB assay format
IgG: immuno-gamma-globulin

IH: immobilised 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
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
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
PP: precision profile
PT: pinch test
PQL: practical quantitation limit
RSA: rabbit serum albumin
RIA: radioimmunoassay
SD: standard deviation of the mean
SPE: solid phase extraction
2,4,5-T: 2,4,5-trichloro-phenoxyacetic acid
TFZ: thiabendazole
TYG: thyroglobulin
UCLA: University of California

REFERENCES

1. Tye, R.J. and Waite, W.M. (1981). Mutagens, carcinogens and the water cycle. Water Pollut. Control : 600-613.

2. Cairns, J. and Mount, D.I. (1990). Aquatic toxicology. Environ. Sci. Technol. 24: 154-161.

3. Bedding, N.D., McIntyre, A.E., Perry, R. and Lester, J.N. (1982). Organic contaminants in the aquatic environment 1. sources and occurrence. Sci. Total Environ. 25: 143-167.

4. Belluck, D.A. and Benjamin, S.L. (1990). Pesticides and human health; defining acceptable and unacceptable risks. J. Environ. Health 53: 11-13.

5. Feliciano, D.V. (1980). Toxics--truth and consequences. J. WPCF 52: 1857-1864.

6. Stara, J.F., Kello, D. and Durkin, P. (1980). Human health hazards associated with chemical contamination of aquatic environment. Environ. Health Perspec. 34: 145-158.

7. Ames, B.N. and Gold, L.S. (1989). Pesticides, risk , and applesauce. Science 244: 755-757.

8. Neal, R.A. (1990). Assessing toxicity of drinking water contaminants: an overview. JAWWA : 44-47.

9. Ames, B.N. and Gold, L.S. (1990). Carcinogens and Human health: Part 1; response. Science 250: 1645-1646.

10. Lijinsky, W. (1989). Environmental cancer risks-real and unreal. Environ. Res. 50: 207-209.

11. Stevens, J.B. and Swackhamer, D.L. (1989). Environmental pollution: a multimedia approach to modelling human exposure. Environ. Sci. Technol. 23: 1180-1186.

12. Wegman, R.C.C. and Melis, P.H.A.M. (1986) Organic pollutants in water. CRC Critical Rev. in Anal. Chem. 16: 281-321.

13. D'Elia, C.F., Sanders, J.G. and Capone, D.G. (1989). Analytical chemistry for environmental sciences: a question of confidence. Environ. Sci. Technol. 23: 768-774.

14. MacCarthy, P., Klusman, R.W. and Rice, J.A. (1989). Water analysis. Anal. Chem. 61: 269R-304R.

15. Sherma, J. (1989). Pesticides. Anal. Chem. 61: 153R-165R.

16. Frehse, H. Trends in pesticide residue methodology. In: Pesticide Science and Biotechnology, eds. R. Greenhalgh and T.R. Roberts; Blackwell, Oxford pp. 293- 300 1987.

17. McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc. Nat. Acad. Sci. USA 72: 5135-5139.

18. Trevors, J.T. Bacterial growth and activity as indicators of toxicity. In: Toxicity Testing Using Microorganisms; Volume 1; eds. G. Bitton and B.J. Dutka; CRC Press Inc., Florida; pp.9-25 1986.

19. Lijinsky, W. (1990). Non-genotoxic environmental carcinogens. Envir. Carcino. Revs. (J.Envir.Sci.Hlth) C8(1): 45-87.

20. Auletta, A. (1990). Current status of short term tests for carcinogenicity. Envir. Car. Revs.(J.Envir.Sci.Hlth.) C8(1): 1-43.

21. Meier, J.R. and Daniel, F.B. (1990). The role of short term tests in evaluating health effects associated with drinking water. JAWWA : 48-56.

22. Mason, J.M., Langenbach, R., Shelby, M.D., Zeiger, E. and Tennant, R.W. (1990). Ability of short-term tests to predict carcinogenesis in rodents. Ann. Rev. Pharmacol. Toxicol. 30: 149-168.

23. Kinae, N., Hahizume, T., Makita, T., Tomita, I., Kimura, I. and Kanamori, H. (1981). Studies on the toxicity of pulp and paper mill effluents-1. Mutagenicity if the sediment samples derived from kraft paper mills. Water Res. 15: 17-24.

24. Hooftman, R.N. and Vink, G.J. (1981). Cytogenetic effects on the Eastern Mudminnow, Umbra pygmaea, exposed to ethyl methanesulfonate, Benzo[a]pyrene, and river water. Ecotoxicol. and Environ. Safety 5: 261-269.

25. Klekowski, E. and Levin, D.E. (1979). Mutagens in a river heavily polluted with paper recycling wastes: results of field and laboratory mutagen assays. Environ. Mutagen. 1: 209-219.

26. Cairns Jr., J. and van der Schalie, W.H. (1980). Biological monitoring Part 1- Early warning systems. Water Research 14: 1179-1196.

27. Bitton, G. and Koopman, B. Biochemical tests for toxicity screening. In: Toxicity Testing Using Microorganisms; eds. G. Bitton and B.J. Dutka; CRC Press Inc., Florida; pp.27-55 1986.

28. Dutka, B.J., Jones, K., Kwan, K.K. and McInnis, R. (1988). Use of microbial and toxicant screening tests for priority site selection of degraded areas in water bodies. Wat. Res. 22: 503-510.

29. Burkhard, L.P. and Ankley, G.T. (1989). Identifying toxicants: NETAC's toxicity-based approach. Environ. Sci. Technol. 23: 1438-1443.

30. Samolloff, M.R., Bell, J., Birkholz, D.A., Webster, G.R.B., Arnott, E.G., Pulak, R. and Madrid, A. (1983). Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. Environ. Sci. Technol. 17: 329-334.

31. Klausner, A. (1987). Immunoassays flourish in new markets. Biotechnol. 5: 551-556.

32. Centeno, E.R., Johnson, W.J. and Sehon, A.H. (1970). Antibodies to two common pesticides, DDT and malathion. Int. Arch. Allergy 37: 1-13.

33. Haas, G.J. and Guardia, E.J. (1968). Production of antibodies against insecticide-protein conjugates. Soc. Exper. Biol. Med. 129: 546-551.

34. Ercegovich, C.D. Analysis of pesticide residues: immunological techniques. In: Pesticide Identification at the Residue Level (R.F. Gould ed.); Advances in Chemistry Series #104, ACS Washington. pp. 162-177. 1971.

35. Langone, J.L. and Van Vunakis, H. (1975). Radioimmunoassay for dieldrin and aldrin. Res. Commun. Chem. Pathol. Pharmacol. 10: 163-171.

36. Lukens, H.R., Williams, C.B., Levison, S.A., Dandliker, W.B., Murayama, D. and Baron, R.L. (1977). Fluorescence immunoassay technique for detecting organic environmental contaminants. Environm. Sci. & Technol. 11: 292-297.

37. Albro, P.W., Luster, M.I., Chae, K., Chaudhary, S.K., Clark, G., Lawson, L.D., Corbett, J.T. and McKinney, J.D. (1979). A radioimmunoassay for chlorinated dibenzo-p-dioxins. Toxicol. Appl. Pharmacol. 50: 137-146.

38. Luster, M.I., Albro, P.W., Chae, K., Lawson, L.D., Corbett, J.D. and McKinney, J.D. (1980). Radioimmunoassay for quantitation of 2,3,7,8-tetrachlorodibenzofuran. Anal. Chem. 52: 1497-1500.

39. Luster, M.I., Albro, P.W., Clark, G., Chae, K., Chaudhary, S.K., Lawson, L.D., Corbett, J.T. and McKinney, J.D. (1979). Production and characterization of antisera specific for chlorinated biphenyl species: initiation of a radioimmunoassay for aroclors. Toxicol. Appl. Pharmacol. 50: 147-155.

40. Hemingway, R.J., Aharonson, N., Greve, P.A., Roberts, T.R. and Thier, H.P. (1984). Improved cost-effective approaches to pesticide residues analysis. Pure & Appl. Chem. 56: 1131-1152.

41. McKinney, J.D., Albro, P.W., Cox, R.H., Hass, J.R. and Walters, D.B. Problems and Pitfalls in Analytical Studies in Toxicology. In: The Pesticide Chemist and Modern Toxicology. Eds. S.K. Bandel, G.J. Marco, L. Goldberg, and M.L. Long. ACS Symposium Series, Chap. 25, pp.439-460. 1981.

42. Cheung, P.Y.K., Gee, S.J. and Hammock, B.D. Pesticide immunoassay as a biotechnology. In: The Impact of Chemistry on Biotechnology; M. Phillips, S.P. Shoemaker, and R.M. Ottenbrite eds. ACS Symposium Series #362 ACS Washington D.C. pp. 217-229. 1988.

43. Hammock, B.D. and Mumma, R.O. Potential of immunochemical technology for pesticide analysis. In: Recent Advances in Pesticide Analytical Methodology. Ed. J. Harvey Jr. and G. Zewig, American Chemical Society Publications, Washington D.C., pp.321-352. 1980.

44. Hammock, B.D., Gee, S.J., Cheung, P.Y.K., Miyamoto, T., Goodrow, M.H., Van Emon, J. and Seiber, J.N. Utility of immunoassay in pesticide trace analysis. In: Pesticide Science and Biotechnology; R. Greenhalgh and T.R. Roberts eds., pp. 309-316, Blackwell Scientific, Ottawa 1987.

45. Newsome, W.H. (1986). Potential and advantages of immunochemical methods for analysis of foods. J. Assoc. Off. Anal. Chem. 69: 919-923.

46. Schwalbe-Fehl, M. (1986). Immunoassays in environmental analytical chemistry. Intern. J. Environ. Anal. Chem. 26: 295-304.

47. Mumma, R.O. and Brady, J.F. Immunological assays for agrochemicals. In: Pesticide Science and Biotechnology, R. Greenhalgh and T.R. Roberts eds., Blackwell Scientific, Ottawa, pp. 341-348. 1987.

48. Hermann, B.W. Immunoassay of pesticides. In: Immunological Techniques in Insect Biology. Eds., L.I. Gilbert and T.A. Miller, Springer-Verlag, New York pp. 135-179, 1988.

49. Hammock, B.D. Applications of immunochemistry in crop protection and biotechnology. In: Biotechnology for Crop Protection; Eds.: P.A. Hedin, J.J. Menn, and R.M. Hollingworth, ACS Symposium Series 379, pp. 298-305, 1988.

50. Harrison, R.O., Gee, S.J. and Hammock, B.D. Immunochemical methods of pesticide residue analysis. In: Biotechnology for Crop Protection. Eds.: P.A. Hedin, J.J. Menn, and R.M. Hollingworth; ACS Symposium Series 379; pp. 316-330. 1988.

51. Vanderlaan, M., Watkins, B.E. and Stanker, L. (1988). Environmental monitoring by immunoassays. Environ. Sci. Technol. 22: 247-254.

52. Jung, F., Gee, S.J., Harrison, R.O., Goodrow, M.H., Karu, A.E., Li, Q.X., Braun, A.L. and Hammock, B.D. (1989). Use of immunochemical techniques for the analysis of pesticides. Pestic. Sci. 26: 303-317.

53. Safe, S. (1986). Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. Ann. Rev. Pharmacol. Toxicol. 26: 371-399.

54. Fukal, L., Rauch, P. and Kas, J. (1988). Use of immunochemical methods in food production chemistry: 1. determination of non-immunogenic low molecular weight compounds. Chem. Listy. 82: 959-977.

55. Haberer, K. and Kramer, P. (1988). Availability of immunochemical detection methods for pesticides in water. Vom Wasser 71: 231-244.

56. Kaufman, B.M. and Clower, Marion, Jr. (1991). Immunoassay of pesticides. J. Assoc. Off. Anal. Chem. 74: 239-247.

57. Wing, K.D. and Hammock, B.D. (1980). Immunochemical methods to detect pesticide residues. Cal. Agric. 34: 34-35.

58. Aherne, G.W. (1985). Immunoassays in the analysis of water. Intern. J. Environ. Anal. Chem. 21: 79-88.

59. Seiber, J.N. and Agrochemistry: An Introduction. IN: The Impact of Chemistry on Biotechnology; M. Phillips, S.P. Shoemaker, R.D. Middlekauf eds. ACS Symposium Series #362 ACS Washington; pp. 204-206, 1988.

60. Hock, B. (1989). Enzyme immunoassay for the determination of pesticides in water. Z. Wasser Abwasser Forsch 22: 78-84.

61. Wraith, M.J. and Britton, D.W. Immunochemical methods for pesticide residue analysis. In: British Crop Protection Conference-Pests and Diseases, V1, pp. 131-136, British Crop Protection council, London 1988.

62. Mumma, R.O. and Hunter, K.W., Jr. Potential of immunoassays in monitoring pesticide residues in foods. In: Pesticide residues in foods: technologies for detection, p.171-181, Congress of the U.S., Office of Technology Assessment 1988.

63. Yalow, R.S. and Berson, S.A. (1959). Assay of plasma insulin in human subjects by immunological methods. Nature 184: 1648-1649.

64. Ekins, R. Merits and disadvantages of different labels and methods of immunoassay. In: Immunoassays for the 80s, Eds. A. Voller, A. Bartlett, and D. Bidwell, University Park Press, Baltimore, pp. 5-16. 1981.

65. Hunter, W.M. Radioimmunoassay. In: Handbook of Immunology. Edited by D.M. Weir. Blackwell Sci. Publ. Oxford. pp. 17.1-17.36. 1973.

66. D.J. McCormick and H.E. Schmitz. Radioimmunoassay. In: Molecular Immunology (M.Z. Atassi, C.J. Van Oss, and D.R. Absolom eds.), Chap. 19, pp. 56-98. Marcel Dekker, New York. 1984.

67. Midgely, A.R. and Niswender, G.D. (1970). Radioimmunoassay of steroids. Acta Endocrine. 147: 320-331.

68. Chapman, D.I. (1979). Radioimmunoassay. Chem. in Britain 15: 439-446.

69. Clifford, M.N. The history of immunoassays in food analysis. In:Immunoassays in Food Analysis; eds. B.A. Morris and M.N. Clifford; Elsevier, London, pp. 3-19, 1985.

70. Morgan, M.R.A. (1989). Mycotoxin immunoassays (with special reference to ELISAs). Tetrahedron 45: 2237-2249.

71. Tonegawa, S. (1985). The molecules of the immune system. Scientific American 253: 122-131.

72. Galfre, G. and Butcher, G.W. Making antibodies. In: Immunology in Plant Science; ed. T.L. Wang, Cambridge University Press, Cambridge; pp. 1-25, 1986.

73. Harlow, E. and Lane, D. Antibodies: A Laboratory Manual. Cold Spring Harbour Laboratory, NY. p. 726, 1988.

74. Coghlan, A. (1991). A second chance for antibodies. New Scientist 129: 34-39.

75. Thompson, M. and Krull, U.J. (1991). Biosensors and the transduction of molecular recognition. Anal. Chem. 63: 393A-405A.

76. Tijssen, P. Practice and theory of enzyme immunoassays. (Laboratory techniques in biochemistry and molecular biology; v.15). Elsevier, Amsterdam. p. 549. 1985.

77. Lankow, R.K., Grothaus, G.D. and Miller, S.A. Immunoassays for crop management systems and agricultural chemistry. In: Biotechnology in Agricultural Chemistry; eds. H. M. LeBaron, R.O. Mumma, R.C. Honeycutt, J.H. Duesing, J.F. Phillips, and M.J. Haas; ACS Symposium Series #334, ACS Washington, DC; pp.228-252, 1987.

78. Van Oss, C.J. and Absolom, D.R. Nature and thermodynamics of antigen-antibody interactions. In: Molecular Immunology; Eds: M.Z. Atassi, C.J. van Oss, and D.R. Absolom, Marcel Dekker Inc., N .Y. pp. 337-360, 1984.

79. Skelley, D.S., Brown, L.P. and Besch, P.K. (1973). Radioimmunoassay. Clin. Chem. 19: 146-186.

80. Morris, B. A. Principles of immunoassay. In: Immunoassays in Food Analysis; eds. B.A. Morris and M.N. Clifford; Elsevier, London; pp. 21-51, 1985.

81. Corrie, J.E.T. Production of anti-hapten sera in rabbits. In: Immunoassays for Clinical Chemistry; Eds. W.M. Hunter and J.E.T. Corrie; Churchill Livingstone, Edinburgh; pp. 469-472, 1983.

82. Landsteiner, K. The specificity of serological reactions. Harvard University Press, Cambridge, MA, 330 pp. 1945.

83. Parker, C.W. Radioimmunoassay of Biologically Active Compounds. Foundations of Immunology Series, Prentice Hall Inc., Englewood Cliffs N.J. p.239, 1976.

84. Warren, J.T. and Gilbert, L.I. Radioimmunoassay: Ecdysteroids. In:Immunological techniques in Insect Biology; Eds. L.I. Gilbert and T.A. Miller; Springer Verlag, New York; pp.181-214, 1988.

85. Erlanger, B.F. The preparation of antigenic hapten-carrier conjugates: a survey. In: Methods in Enzymology 70: Immunochemical Techniques; Eds., H. Van Vunakis and J.J. Langone; Acad. Press, New York, pp. 85-151, 1980.

86. Weiler, E.W., Eberle, J., Mertens, R., Atzorn, R., Feyerabend, M., Jourdan, P.S., Arnscheidt, A. and Wieczorek, U. Antisera - and monoclonal antibody-based immunoassay of plant hormones. In: Immunology in Plant Science; ed. T.L. Wang; Cambridge University Press; Cambridge, pp. 27-58, 1986.

87. Kelley, M.M., Zahnow, E.W., Petersen, W.C. and Toy, S.T. (1985). Chlorosulfuron determination in soil extracts by enzyme immunoassay. J. Agric. Food Chem. 33: 962-965.

88. Erlanger, B.F., Beiser, S.M., Borek, Felix, Edel, F. and Lieberman, S. The preparation of steroid-protein conjugates to elicit antihormonal antibodies. In: Methods in Immunology and Immunochemistry. Eds. C.A. Williams and M.W. Chase. Vol.1. Academic Press, New York pp. 144-150, 1967.

89. Bauminger, S. and Wilchek, M. The use of carbodimides in the preparation of immunizing conjugates. In: Methods in Enzymology 70:Acad. Press New york; pp. 151-159, 1980.

90. Safe, S. (1984). Polychlorinated biphenyls (PCBS) and polybrominated biphenyls (PBBS): biochemistry, toxicology, and mechanism of action. Critic. Rev. Toxicol. 13: 319-395.

91. Nisonoff, A. Conjugated and Synthetic Antigens. In: Methods in Immunology and Immunochemistry. Ed. C.A. Williams and M.W. Chase Vol. 1 Preparation of Antigens and Antibodies. Acad. Press New York and London. 1967.

92. Butt, W.R. Introduction and Overview. In: Practical Immunoassay; Ed. W.R. Butt, Marcel Decker, Inc., New York, pp. 1-17, 1984.

93. Hurn, B.A. and Chantler, S.M. Production of reagent antibodies. In:Methods in Enzymology. Eds. H. van Vunakis and J.J. Langone. Academic Press, New York-London, pp. 104-141, 1980.

94. Bankert, R.B., Jou, Y. and Mayers, G.L. Monoclonal antibodies/myelomas and hybridomas. IN: Molecular Immunology. Eds: M.Z. Atassi, C.J. van Oss, and D.R. Absolom. Marcel Dekker, N.Y., pp.175-200, 1984.

95. Ling, N.R. and Jefferis, R. Monoclonal antibodies. In: Practical Immunoassay; Ed. W.R. Butt; Marcel Dekker, New York; pp. 199-215, 1984.

96. Campbell, A.M. Monoclonal Antibody Technology. Elsevier, Amsterdam, Netherlands. p. 265., 1984.

97. Vanderlaan, M., Stanker, L.H., Watkins, B.E., Petrovic, P. and Gorbach, S. (1988). Improvement and application of an immunoassay for screening environmental samples for dioxin contamination. Environ. Toxicol. and Chem. 7: 859-870.

98. Bolton, A.E. Radioimmunoassay. In: Immunoassays for the 80s, Eds. A. Voller, A. Bartlett, and D. Bidwell, University Park Press, Baltimore. pp. 69-83, 1981.

99. Dakubu, S., Ekins, R., Jackson, T. and Marshall, N.J. High sensitivity, pulsed-light time-resolved fluoroimmunoassay. In: Practical Immunoassay; Ed. W.R. Butt; Marcel Decker, Inc., pp.71-101, 1984.

100. Chard, T. An introduction to radioimmunoassay and related techniques. Elsevier, Amsterdam m; p.274, 1987.

101. Bolton, A.E. Radioiodination techniques. Review 18; Amersham Corporation, Arlington Heights, Illinois; p.71, 1978.

102. Walker, W.H.C. (1977). An approach to immunoassay. Clin. Chem. 23: 384-402.

103. Jeffcoate, S.L., Edwards, R., Gilby, E.D. and White, N. The use of [H-3]-labelled ligands in steroid radioimmunoassays. In: Steroid Immunoassay; Proceedings of 5th Tenovus Workshop, Cardiff, April 1974. Eds. E.H.D. Cameron, S.G. Hillier, and K. Griffiths, Alpha Omega Publishing, Cardiff; pp. 133-140, 1975.

104. Hunter, W.M., Nars, P.W. and Rutherford, F.J. Preparation and behaviour of 125I-labelled radioligands for phenolic and neutral steroids. In: Steroid Immunoassay; Proceedings of 5th Tenovus Workshop; pp. 141-152, Cardiff, 1974; Eds. E.H.D. Cameron, S.G. Hillier, and K. Griffiths; Alpha Omega Publishing, Cardiff 1975.

105. Butt, W.R. Problems of iodination. In: Practical Immunoassay; Ed. W.R. Butt, Marcel Decker, New York; pp. 19-35, 84.

106. Hawker, C.D. (1973). Radioimmunoassay and related methods. Anal. Chem. 45: 878A-890A.

107. Cameron, E.H.D., Scarisbrick, J.J., Morris, S.E. and Read, G. 125I-iodohistamine derivatives as tracers for the radioimmunoassay of progestagens. In: Steroid Immunoassay, Proceedings of the 5th Tenovus Workshop, Cardiff, April 1974; Eds. E.H.D. Cameron, S.G. Hillier, and K. Griffiths; Alpha Omega Publishing, Cardiff; pp.153-164, 1975.

108. Ekins, R.P. Discussion on p. 172 of Steroid Immunoassay. Proceedings of fifth Tenovus Work shop, Eds: E.H.D. Cameron, S.G. Hillier, and K. Griffiths. Cardiff Alpha Omega Publishing, 1975.

109. Nordblom, G.D., Webb, R., Counsell, R.E. and England, B.G. (1981). A chemical approach to solving bridging phenomena in steroid radioimmunoassays. Steroids 38: 161-173.

110. BioNebraska Inc. Personal communication. 1991.

111. Swinkels, L.M.J.W., Ross, H.A. and Benraad, Th. J. (1991). Scintillation proximity assay: determination of steroid hormones without separation of antibody bound and free ligand. Ann. Clin. Biochem. 28: 87-90.

112. Chard, T. Ammonium sulfate and polyethylene glycol as reagents to separate antigen from a nitigen-antibody complexes. In: Methods in Enzymology 70: Eds. H. Van Vunakis and J.J. Langone; Acad. Press New York. pp. 280-291, 1980.

113. Collins, W.P., Barnard, G.J.R. and Hennam, J.F. Factors affecting the choice of separation technique. In: Steroid Immunoassay, Proceedings of 5th Tenovus Workshop, Cardiff April 1974; Eds. E.H.D. Cameron, S.G. Hillier, and K. Griffiths, Alpha Omega Publishing, Cardiff; pp. 223-228, 1975.

114. Odell, W.D. Use of charcoal to separate antibody complexes from free ligand in radioimmunoassay. In: Methods in Enzymology, 70: Eds. H. Van Vunakis and J.J. Langone, Acad. Press, New York; pp. 274-279, 1980.

115. A.R. Midgley, Jr. and Hepburn, M.R. Use of the double-antibody method to separate antibody bound from free ligand in radioimmunoassay. In: Methods in Enzymology, 70: Eds. H. Van Vunakis and J.J. Langone; Acad. Press, New York; pp. 266-274, 1980.

116. Odell, W.D., Silver, C. and Grover, P.K. Competitive protein binding assays methods of separation of bound from free. In: Steroid Immunoassays, Proceedings of 5th Tenovus Workshop, Cardiff, April 1974; Eds. E.H.D. Cameron, S.G. Hillier, and K. Griffiths, Alpha Omega Publishing, Cardiff, pp. 207-222, 1975.

117. Gilman, A.P., Douglas, V.M., Newhook, R.C. and Arbuckle, T.E. Chlorophenols and their impurities: a health hazard evaluation. Health and Welfare Canada: 88-EHD-110, p.72, 1988.

118. Ekins, R., Chu, F. and Biggart, E. (1990). Fluorescence spectroscopy and its application to a new generation of high sensitivity, multi-microspot, multianalyte, immunoassay. Clin. Chimica Acta 194: 91-114.

119. Engvall, E. and Perlmann, P. (1971). Enzyme-linked immunoadsorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 8: 871.

120. Van Weeman, B.K., and Schuurs, A.H.W.M. (1971). Immunoassay using antigen-enzyme conjugates. FEBS Lett. 15: 232.

121. Wisdom, G.B. (1976). Enzyme-immunoassay. Clin. Chem. 22: 1243-1255.

122. O'Sullivan, M.J. Enzyme Immunoassay. In: Practical Immunoassay. Ed. W.R. Butt, Marcel Dekker, New York, pp. 37-69, 1984.

123. Sauer, M.J., Foulkes, J.A. and Morris, B.A. Principles of enzyme immunoassay. IN: Immunoassays in Food Analysis. Eds. B.A. Morris and M.N. Clifford; Elsevier, London, pp. 53-72, 1985.

124. Allen, J.C. and Smith, C.J. (1987). Enzyme-linked immunoassay kits for routine food analysis. Tibtech 5: 193-199.

125. Chu, F.S. (1984). Immunoassays for analysis of mycotoxins. J. Food Protect. 47: 562-569.

126. Avrameas, S. Heterogenous enzyme immunoassays. In: Immunoassays for the 80s, Eds. A. Voller , A. Bartlett, and D. Bidwell, University Park Press, Baltimore, pp.85-90. 1981.

127. Walls, K.W. Enzyme immunoassays. In: Molecular Immunology. Eds. Atassi, M.Z., Van Oss, C.J . Absolom, D.R. Marcel Dekker, New York, New York p.725, 1984.

128. Morgan, M.R.A., McNerney, R. and Chan, H. W.-S. An ELISA for the analysis of the mycotoxin Ochratoxin A in food. In: Immunoassays in Food Analysis; eds. B.A. Morris and M.N. Clifford; Elsevier, London; pp. 159-167, 1985.

129. Landon, J. and Kamel, R.S. Immunoassays employing reactants labelled with a fluorophore. In: Immunoassays for the 80s; eds. A. Voller, A. Bartlett, and D. Bidwell; University Park Press Baltimore; pp. 91-112, 1981.

130. Diamandis, E.P. and Christopoulos, T.K. (1990). Europium chelate labels in time-resolved fluorescence immunoassays and DNA hybridization assays. Anal. Chem. 62: 1149A-1157A.

131. Olsson, T. and Thore, A. Chemiluminescence and its use in immunoassay. In: Immunoassays for the 80s; eds. A. Voller, A. Bartlett, D. Bidwell, University Park Press, Baltimore; pp. 113-125, 1981.

132. Weeks, I., Campbell, A.K., Woodhead, J.S. and McCapra, F. Immunoassays using chemiluminescent labels. IN: Practical Immunoassay: The State of the Art; ed. W.R. Butt, Marcel Dekker Inc. New York; pp.103-116, 1984.

133. Katz, S.E. and Brady, M.S. (1990). High-performance immunoaffinity chromatography for drug residue analysis. J. Assoc. Off. Anal. Chem. 73: 557-560.

134. Kimbrough, 1990. (1990). How toxic is 2,3,7,8-tetrachlorodibenzodioxin to humans?. J. Toxicol. Environ. Health 30: 261-271.

135. Wolfe, W.H., Miner, J.C. and Michalek, J.E. An epidemiologic investigation of health effects in air force personnel following exposure to herbicides: serum dioxin analysis of 1987 examination results. Presented at Dioxin '91. Abstract S77, Sept. 23-27, Raleigh-Durham, N.C. USA, 1991.

136. 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. and Suruda, A.J. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Presented at Dioxin 91. Abstract S78. September 23-27, Raleigh-Durham, NC, USA, 1991.

137. McKinney, J., Albro, P., Luster, M., Corbett, B., Schroeder, J. and Lawson, L. Development and reliability of a radioimmunoassay for 2,3,7,8-tetrachlorodibenzo-p-dioxin. In: Chlorinated Dioxins and Related Compounds. Ed. O. Hutzinger et al. Pergamon Press Oxford and New York, pp. 67-75, 1982.

138. Bright, F.V., Betts, T.A. and Litwiler, K.S. (1990). Regenerable fibre-optic-based immunosensor. Anal. Chem. 62: 1065-1069.

139. Albro, P.W., Luster, M.I., Chae, K., Clark, G. and McKinney, J.D. (1982). Radioimmunoassay of chlorinated dibenzo-p-dioxins. Methods Enzymol. 84: 619-628.

140. Sherry, J.P., ApSimon, J.W., Collier, T.L. and Albro, P.W. (1990). Use of dimethyl sulfoxide as solubilization agent in the detection of 2,3,7,8-T CDD by radioimmunoassay. Chemosphere 20: 1409-1416.

141. Sherry J.P., ApSimon, J., Collier, T., Afghan, B. and Albro, P. (1989). The use of radioimmunoassay for the detection of PCDDs in fish samples. Chemosphere 19: 255-261.

142. Collier, T.L., ApSimon, J.W. and Sherry, J.P. (1990). Development of an improved hapten for use in the radioimmunoassay of dioxins. Chemosphere 20: 301-308.

143. Collier, T.L. 1989. Synthesis of Dioxins as Analytical and Toxicological Standards. Ph.D. Thesis, Carleton University, Ottawa.

144. Sherry, J.P. and Albro, P.W. Radioimmunoassay of chlorinated dioxins: use of [3H]-labelled 2,3,7,8-tetrachlorodibenzo-p-dioxin as radioligand. Presented at Dioxin '90 Bayreuth, Germany. September 1990.

145. Kennel, S.J., Jason, C., Albro, P.W., Mason, G. and Safe, S.H. (1986). Monoclonal antibodies to chlorinated dibenzo-p-dioxins. Toxicol. Appl. Pharmacol. 82: 256-263.

146. Stanker, L., Watkins, B., Vanderlaan, M. and Budde, W.L. (1987). Development of an immunoassay for chlorinated dioxins based on a monoclonal antibody and an enzyme linked immunosorbent assay (ELISA). Chemosphere 16: 1635-1639.

147. Stanker, L.H., Watkins, B., Rogers, N. and Vanderlaan, M. (1987). Monoclonal antibodies for dioxin: antibody characterization and assay development. Toxicology 45: 229-243.

148. Vanderlaan, M., Van Emon, J., Watkins, B. and Stanker, L. Monoclonal antibodies for the detection of trace chemicals. In: Pesticide Science and Biotechnology. Greenhalgh, R. and T.R. Roberts eds.; Blackwell Scientific, Ottawa pp. 597-602, 1987.

149. Bolts, J.M., Diamond, S.E., Kolc, J.F., Lin, S.H., Regina, F.J., Koga, P.G.,Misener, G.C., Schmidt, J.C. and Integrated immunochemical systems for environmental monitoring. In: First International Symposium on Field Screening Methods for Hazardous Waste Site Investigations; Oct. 11-13, Las Vegas,; pp. 243-247, 1988.

150. Nakamura, N. and Matsunaga, T. (1991). Fibre optic sensor with a sandwich binding technique for fluoroimmunoassay. Anal. Letters 24: 1075-1084.

151. IARC monograph #18, p. 43, Lyon 1978, cited in 19.

152. Silberhorn, E.M., Glauert, H.P. and Robertson, L.W. (1990). Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. Critic. Rev. Toxicol. 20: 439-496.

153. Kusterbeck, A.W., Wemhoff, G.A., Charles, P.T., Yeager, D.A., Brederhorst, R., Vogel, C.-W. and Ligler, F.S. (1990). A continuous flow immunoassay for rapid and sensitive detection of small molecules. J. Immunol. Methods 135: 191-197.

154. Newsome, W.H. and Shields, J.B. (1981). Radioimmunoassay of PCBs in milk and blood. Intern. J. Environ. Anal. Chem. 10: 295-304.

155. Chamerlik-Cooper, M.A., Carlson, R.E. and Harrison, R.O. Determination of PCBs in soil by enzyme immunoassay. Presented at Second International Symposium, Field Screening Methods for Hazardous Waster Site Investigations, US EPA, Las Vegas, February, 1991.

156. Johnson, H.J., Cernosek, S.F., Gutierrez-Cernosek, R.M. and Brown, L.L. (1980). Development of a radioimmunoassay procedure for 4-acetamidobiphenyl, a metabolite of the chemical carcinogen 4-aminobiphenyl, in urine. J. Anal. Toxicol. 4: 86-90.

157. Johnson, H.J., Cernosek, S.F., Gutierrez-Cernosek, R.M. and Brown, L.L. (1981). Validation of a radioimmunoassay procedure for N,N'-diacetylbenzidine, a metabolite of the chemical carcinogen benzidine, in urine. J. Anal. Toxicol. 5: 157-161.

158. Gomes, M. and Santella, R. (1990). Immunologic methods for the detection of benzo-a-pyrene metabolites in urine. Chem. Res. Toxicol. 3: 307-310.

159. Leginus, J. Westinghouse Bio-analytic Systems Company, Pentachlorophenol immunoassay performance data. Personal Communication 1989.

160. Rinder, D.F. and Fleeker, J.R. (1981). A radioimmunoassay to screen for 2,4-dichlorophenoxyacetic acid and 2,4,5-trichloroacetic acid in surface water. Bull. Environm. Contam. Toxicol. 26: 375-380.

161. Fleeker, J. (1987). Two enzyme immunoassays to screen for 2,4-dichlorophenoxyacetic acid in water. J. Assoc. Off. Anal. Chem. 70: 874-878.

162. Knopp, D., Nuhn, P. and Dobberkau, H.-J. (1985). Radioimmunoassay for 2,4-dichlorophenoxyacetic acid. Arch. Toxicol. 58: 27-32.

163. Knopp, D., Nuhn, P. and Mittag, E. (1986). Preparation of radioactively labelled tracers of 2,4,-dichlorophenoxyacetic acid for radioimmunoassay. Pharmazie 41: 143.

164. Hall, J.C., Deschamps, R.J.A. and Kreig, K.K. (1989). Immunoassays for the detection of 2,4,-D and picloram in river water and urine. J. Agric. Food Chem. 37: 981-984.

165. Dunbar, B.D., Niswender, G.D. and Hudson, J.M. (1985). Antibody for the detection and quantification of atrazine. United States Patent 4,530,786: 1-6.

166. Huber, S.J. (1985). Improved solid-phase enzyme immunoassay systems in the ppt range for atrazine in fresh water. Chemosphere 14: 1795-1803.

167. Dunbar, B., Riggle, B. and Niswender, G. (1990). Development of enzyme immunoassay for the detection of triazine herbicides. J. Agric. Food Chem. 38: 433-437.

168. Wittmann, C. and Hock, B. (1989). Improved enzyme immunoassay for the analysis of s-triazines in water samples. Food Agric. Immunol. 1: 211-224.

169. Sharp, J.K., Robotti, K.M., Ehrmann, P.R., Brown, L.J. and Hermann, B.W. Quantitative analysis of cyanazine in groundwater and soil by enzyme-linked immunoassay. Cited in Hermann, 1988, 1986.

170. Robotti, K.M., Sharp, J.K., Ehrmann, P.R., Brown, L.J. and Hermann, B.W. An ELISA method for the detection of cyanazine. In: Abstracts of Papers, 192nd ACS National Meeting. ACS, Washington DC. AGRO 42, 1986.

171. Van de Water, C. and Haagsma, N. (1990). A sensitive streptavidin-biotin enzyme-linked immunosorbent assay for rapid screening of residues of chloramphenicol in milk. Food Agric. Immunol. 2: 11-19.

172. Huber, S.J. and Hock, B. (1985). A solid phase enzyme immunoassay for quantitative determination of the herbicide terbutryn. J. Plant Diseases Protect. 92: 147-156.

173. Huber, S.J. and Hock, B. (1985). Solid-phase enzyme immunoassay for the detection of herbicides in fresh water - polystyrene beads as antibody carriers compared to microtitre plates. GIT Fachz. Lab. 10: 969-977.

174. Wuest, S., Doht, U., Giersch, T., Wittmann, C. and Hock, B. (1990). Sensitive s-triazine enzyme immunoassay for water samples in polystyrene tubes. GIT Fachz. Lab. 34: 99-106.

175. Wittmann, C. and Hock, B. (1991). Development of an ELISA for the analysis of atrazine metabolites deethylatrazine and deisopropylatrazine. J. Agric. Food. Chem. 39: 1194-1200.

176. Aherne, G.W. (1990). Enhanced luminescent immunoassays for environmental monitoring. Anal. Proc. 27: 100-101.

177. Hardcastle, A., Aherne, W. and Marks, V. (1988). A sensitive enhanced luminescent immunoassay for the triazine pesticides atrazine and simazine. J. Biolumin. Chemilumin. 2: 209.

178. Seare, N.J., Miller, J.N., Cole, E.R., Samuel, A.J. and Woodbridge, A.P. (1986). Fluorescence immunoassay in pesticide analysis. Anal. Proc. 23: 220.

179. Bushway, R.J., Perkins, B., Savage, S.A., Lekousi, S.J. and Ferguson, B.S. (1988). Determination of atrazine residues in water and soil by enzyme immunoassay. Bull. Environ. Contam. Toxicol. 40: 647-654.

180. Schulze, P.A. and Capel, P.D. Utility and limitations of immunoassay tests as a field screening method for triazine herbicides. Presented at "US Geological Survey 2nd National Symposium On Water Quality". Orlando. Florida, Nov. 12 - 17, 1989.

181. Schlaeppi, J.-M., Fory, W. and Ramsteiner, K. (1989). Hydroxyatrazine and atrazine determination in soil and water by enzyme linked immunosorbent assay using specific monoclonal antibodies. J. Agric. Food Chem. 37: 1532-1538.

182. Giersch, T. and Hock, B. (1990). Production of monoclonal antibodies for the determination of s-triazines with enzyme immunoassays. Food Agric. Immunol. 2: 85-97.

183. Goodrow, M.H., Harrison, R.O. and Hammock, B.D. (1990). Hapten synthesis, antibody development, and competitive inhibition enzyme immunoassay for s-triazine herbicides. J. Agric. Food Chem. 38: 990-996.

184. Harrison, R.O., Goodrow, M.H. and Hammock, B.D. (1991). Competitive inhibition ELISA for the s-Triazine herbicides: assay optimisation and antibody characterisation. J. Agric. Food Chem. 39: 122-128.

185. Levitt, T. (1977). Radioimmunoassay for paraquat. Lancet 8033: 358.

186. Levitt, T. (1979). Determinations of paraquat in clinical practice using radioimmunoassay. Proc. Analyt. Div. Chem. Soc. 16: 72-76.

187. Fatori, D. and Hunter, W.M. (1980). Radioimmunoassay for serum paraquat. Clinica Chimica Acta 100: 81-90.

188. Niewola, Z., Walsh, S.T. and Davies, G.E. (1983). Enzyme linked immunosorbent assay (ELISA) for paraquat. Int. J. Immunopharmac. 5: 211-218.

189. Niewola, Z., Hayward, C., Symington, B.A. and Robson, R.T. (1985). Quantitative estimation of paraquat by an enzyme linked immunosorbent assay using a monoclonal antibody. Clin. Chim. Acta 148: 149-156.

190. Benner, J. and Niewola, Z. Paraquat in soil. In: Methods of Enzymatic Analysis, Eds.: J. Bergmeyer and M. Grabl, VCH Verlagsgesellschaft, Weinheim (FRG) pp.451-464 1986.

191. Horstmann, B.J., Chase, H.A. and Kenney, C.N. (1990). Purification of anti-paraquat monoclonal antibodies by affinity chromatography on immobilized hapten. J. Chromatogr. 516: 433-441.

192. Nagao, M., Takatori, T., Wu, B., Terazawa, K., Gotouda, H. and Akabane, H. (1989). Development and characterization of monoclonal antibodies reactive with paraquat. J. Immunoassay 10: 1-17.

193. Van Emon, J.M., Seiber, J.N. and Hammock, B.D. Applications of immunoassay to paraquat and other pesticides. In: Bioregulators for Pest Control; Ed. P.A. Hedin; ACS Symposium Series #276, ACS Washington, D.C. pp. 307-316, 1985.

194. Van Emon, J., Hammock, B.D. and Seiber, J.N. (1986). Enzyme-linked immunosorbent assay for paraquat and its application to exposure analysis. Anal. Chem. 58: 1866-1873.

195. Van Emon, J., Seiber, J. and Hammock, B. (1987). Application of an enzyme-linked immunosorbent assay (ELISA) to determine paraquat residues in milk, beef, and potatoes. Bull. Emviron. Contam. Toxicol. 39: 490-497.

196. Schwalbe, M., Dorn, E. and Beyermann, K. (1984). Enzyme immunoassay and fluoroimmunoassay for the herbicide diclofop-methyl. J. Agric. Food Chem. 32: 734-741.

197. Koppatschek, F.K., Liebl, R.A., Kriz, A.L. and Melhado, L.L. (1990). Development of an enzyme-linked immunosorbent assay for the detection of the herbicide clomazone. J. Agric. Food Chem. 38: 1519-1522.

198. Dargar, R.V., Tymonko, J.M. and VanDerWerf, P. (1991). Clomazone measurement by enzyme-linked immunosorbent assay. J. Agric. Food Chem. 39: 813-819.

199. Feng, P.C.C., Wratten, S.F., Horton, S.R., Sharp, C.R. and Logusch, E.W. (1990). Development of an enzyme-linked immunosorbent assay for alachlor and its application to the analysis of environmental water samples. J. Agric. Food Chem. 38: 159-163.

200. Schlaeppi, J.-M., Moser, H. and Ramsteiner, K. (1991). Determination of metalochlor by competitive enzyme immunoassay using a specific monoclonal antibody. J. Agric. Food Chem. 39: 1533-1536.

201. Scholz, H.M. and Hock, B. (1991). Development of an enzyme immunoassay for the determination of metazchlor. Anal. Letters 24: 413-427.

202. Harrison, R.O., Brimfield, A.A. and Nelson, J.O. (1989). Development of a monoclonal antibody based enzyme immunoassay for analysis of maleic hydrazide. J. Agric. Food Chem. 37: 958-964.

203. Heineman, W.R. and Halsall, H.B. (1985). Strategies for electrochemical immunoassay. Anal. Chem. 57: 1321A-1331A.

204. Gee S.J., Miyamoto, T., Goodrow, M.H., Buster, D. and Hammock, B.D. (1988). Development of an enzyme-linked immunosorbent assay for the analysis of the thiocarbamate herbicide molinate. J. Agric. Food Chem. 36: 863-870.

205. Deschamps, R.J.A., Hall, J.C. and McDermott, M.R. (1990). Polyclonal and monoclonal enzyme immunoassays for picloram detection in water, soil, plants, and urine. J. Agric. Food Chem. 38: 1881-1886.

206. Jung, F., Szekacs, A., Li, Q. and Hammock, B.D. (1991). Immunoachemical approach to the detection of aminotriazoles using selective amino group protection by chromophores. J. Agric. Food Chem. 39: 129-136.

207. Newsome, W.H. and Collins, P.G. (1991). Determination of imazamethabenz in cereal grain by enzyme-linked immunosorbent assay. Bull. Environ. Contam. Toxicol. 47: 211-216.

208. Li, Q.X., Hammock, B.D. and Seiber, J.N. (1991). Development of an enzyme-linked immunosorbent assay for the herbicide bentazon. J. Agric. Food Chem. 39: 1537-1544.

209. Riggle, B. (1991). Development of a preliminary enzyme-linked immunosorbent assay for the herbicide trifuralin. Bull. Environ. Contamin. Toxicol. 46: 404-409.

210. Riggle, B. and Dunbar, B.D. (1990). Development of enzyme immunoassay for the detection of the herbicide Norflurazo n. J. Agric. Food Chem. 38: 1922-1925.

211. Bushway, R.J., Pask, W.M., King, J., Perkins, B. and Ferguson, B.S. Determination of chlordane in soil by enzyme immunoassay. In: First International Symposium on Field Screening Methods for Hazardous Waste Site Investigation s; Oct. 11-13, Las Vegas 1988.

212. Dreher, R.M. and Podratzki, B. (1988). Development of an enzyme immunoassay for endosulfan and its degradation products. J. Agric. Food Chem. 36: 1072-1075.

213. Frevert, J., Zietz, E. and Knoell, H.E. Residue and groundwater analysis: new techniques. In: Brighton Crop Protection Conference - Pests and Diseases; pp. 727-731 1988.

214. Stanker, L.H., Bigbee, C., Van Emon, J., Watkins, B., Jensen, R.H., Morris, C. and Vanderlaan, M. (1989). An immunoassay for pyrethroids: detection of permethrin in meat. J. Agric. Food. Chem. 37: 834-839.

215. Wing, K.D., Hammock, B.D. and Wustner, D.A. (1978). Development of an s-bioallethrin specific antibody. J. Agric. Food Chem. 26: 1328-1333.

216. Wing, K.D. and Hammock, B.D. (1979). Stereo selectivity of a radioimmunoassay for the insecticide S-bioallethrin. Experientia 35: 1619-1620.

217. Van Emon, J.M., Seiber, J.N. and Hammock, B.D. Application and selectivity of an enzyme-linked immunosorbent assay (ELISA) for the pyrethroid s-bioallethrin. In: Sixth Int. Congress of Pesticide 5C-01 Chemistry 1986.

218. Roberts, T.R. (1985). Current trends in pesticide residue analysis. Trends in Anal. Chem. 4: 3-7.

219. Wraith, M.J., Hitchings, E.J., Woodbridge, A.P., Cole, E.R. and Roberts, T.R. Development of immunoassay methods for pyrethroid insecticides. In: Sixth International Congress of Pesticide Chemistry, 5C-10 1986.

220. Schmid, R.D. and Kunnecke, W. (1990). Flow injection analysis (FIA) - applications in life sciences. J. Biotechnol. 14: 3-31.

221. Kramer, P. and Schmid, R. (1991). Flow injection immunoanalysis (FIIA) - a new immunoassay format for the determination of pesticides in water. Biosensors & Bioelectronics 6: 239-243.

222. Wie, S.I., Sylwester, A.P., Wing, K.D. and Hammock, B.D. (1982). Synthesis of haptens and potential radioligands and development of antibodies to insect growth regulators diflubenzuron and BAY SIR 8514. J. Agric. Food Chem. 30: 943-948.

223. Wie, S.I. and Hammock, B.D. (1982). Development of enzyme-linked immunosorbent assays for residue analysis of diflubenzuron and BAY SIR 8514. J. Agric. Food Chem. 30: 949-957.

224. Wie, S.I. and Hammock, B.D. (1984). Comparison of coating and immunizing antigen structure on the sensitivity and specificity of immunoassays for benzylphenylurea insecticides. J. Agric. Food Chem. 32: 1294-1301.

225. Ercegovich, C.D., Vallejo, R.P, Gettig, R.R., Woods, L., Bogus, E.R. and R. O. Mumma, R.O. (1981). Development of a radioimmunoassay for parathion. J. Agric. Food Chem. 29: 559-563.

226. Vallejo, R.P., Bogus, E.R. and Mumma, R.O. (1982). Effects of hapten structure and bridging groups on antisera specificity in parathion immunoassay development. J. Agric. Food Chem. 30: 572-580.

227. Conaway, J.E. (1991). New trends in analytical technology and methods for pesticide residue analysis. J. Assoc. Off. Anal. Chem. 74: 715-717.

228. Hunter, K.W. and Lenz, D.E. (1982). Detection and quantification of the organophosphate insecticide paraoxon by competitive inhibition enzyme immunoassay. Life Sciences 30: 355-361.

229. Hunter, K.W., Lenz, D.E. and Brimfield, A.A. Paraoxon in body fluids. In: Methods of Enzymatic Analysis. Eds. J. Bergmeyer and M. Grabl, VCH Verlagsgesellschaft, pp. 426-438 1986.

230. Brimfield, A.A., Lenz, D.E., Graham, C. and Hunter, K.W. (1985). Mouse monoclonal antibodies against Paraoxon: potential reagents for immuno- assay with constant immunochemical characteristics. J. Agric. Food Chem. 33: 1237-1242.

231. Heldman, E., Balan, A., Horowitz, O., Ben-Zion, S. and Torten, M. (1985). A novel immunoassay with direct relevance to protection against organophosphate poisoning. FEBS 180: 243-248.

232. Sudi, J. and Heeschen, W. (1988). Studies on the development of an immunoassay for the group specific detection of the dimethyl ester of phosphates, thiophosphates, dithiophosphates and phosphonates. Kieler Milchwirtschaftlich Forschung. 40: 179-203.

233. Duquette, P.H., Guire, P.E. and Swanson, M.J. Fieldable enzyme immunoassay kits for pesticides. In: First International Symposium on Field Screening Methods for Hazardous Waste Site Investigations, October 11-13, 1988, Las Vegas; pp. 239-242 1988.

234. Hunter, K.W., Lenz, D.E., Brimfield, A.A. and Naylor, J.A. (1982). Quantification of the organophosphorous nerve agent soman by competitive inhibition enzyme immunoassay using monoclonal antibody. FEBS Letters 149: 147-151.

235. Lenz, D.E., Brimfield, A.A., Hunter, K.W., Benschop, H.P., De Jong, L.P.A., Van Dijk, C. and Clow, T.R. (1984). Studies using a monoclonal antibody against soman. Fund. Appl. Toxicol. 4: S156-S164.

236. Schmidt, P., Kuhlmann, R. and Losch, U. (1988). A competitive inhibition enzyme immunoassay for detection and quantification of organophosphorous compounds. Z. Naturforsch. 43c: 167-172.

237. Erhard, M.H., Schmidt, P., Kuhlmann, R. and Losch, U. (1989). Development of an ELISA for detection of an organophosphorus compound using mon clonal antibodies. Arch. Toxicol. 63: 462-468.

238. Erhard, M.H., Kuhlmann, R., Szinicz, L. and Losch, U. (1990). Detection of the organophosporous nerve agent soman by an ELISA using monoclonal antibodies. Arch. Toxicol. 64: 580-585.

239. Banerjee, B.D. (1987). Development of an enzyme-linked immunosorbent assay for the quantification of DDA (2,2-bis(p-chlorophenyl) acetic acid) in urine. Bull. Environ. Contam. Toxicol. 38: 798-804.

240. Brady, J.F., Fleeker, J.R., Wilson, R.A. and Mumma, R.O. Enzyme immunoassay for aldicarb. In: ACS symposium Series #382, American Chemical Society Washington DC; p. 262-284 1989.

241. Brady, J.G. Ph.D. Thesis, Pennsylvania State University, University Park, PA. Cited in Jung et al., 1989a, 1988.

242. Wie, S.I., Andrews, Jr., R.E., Hammock, B.D., Faust, R.M. and Bulla, Jr., L.A. (1982). Enzyme-linked immunosorbent assays for detection and quantitation of the entomocidal parasporal crystalline protein of Bacillus thuringiensis subspp. Kurstaki and israelensis. Appl. Environ. Microbiol. 43: 891-894.

243. Wie, S.I., Hammock, B.D., Gill, S.S., Grate, E., Andrews Jr., R.E., Faust, R.M., Bulla Jr., L.A. and Schaefer, C.H. (1984). An improved enzyme-linked immunoassay for the detection and quantification of the entomocidal parasporal crystal proteins of Bacillus thurgiensis subspp. Kurstaki and israelensis. Appl. Environ. Microbiol. 57: 447-454.

244. Cheung, P.Y.K. and Hammock, B.D. Monitoring Bacillus thuringiensis in the environment with enzyme linked immunosorbent assay. In: Biotechnology for Crop Protection; Eds. P.A. Hedin, J.J. Menn, and R.M. Hollingworth; ACS Symposium Series # 379, ACS Washington DC; pp. 35 9-372, 1988.

245. Smith, R.A. and Ulrich, J.T. (1983). Enzyme-linked immunosorbent assay for quantitative detection of Bacillus thurin giensis crystal protein. Appl. Environ. Microbiol. 45: 586-590.

246. Wikman-Coffelt, J. and Berg, H.W. (1976). Radioimmunoassay for the analysis of ethylcarbamate in wine. Am. J. Enol. Vitic. 27: 115-117.

247. Newsome, W.H. and Shields, J.B. (1981). A radioimmunoassay for benomyl and methyl 2-benzimidazolecarbamate on food crops. J. Agric. Food Chem. 29: 220-222.

248. Newsome, W.H. and Collins, P.G. (1987). Enzyme-linked immunosorbent assay of benomyl and thiabendazole in some foods. J. Assoc. Off. Anal. Chem. 70: 1025-1027.

249. Bushway, R.J., Savage, S.A. and Ferguson, B.S. (1990). Determination of methyl 2-benzimidazolecarbamate in fruit juices by immunoassay. Food Chem. 35: 51-58.

250. Newsome, W.H. (1985). An enzyme linked immunosorbent assay for metalaxyl in foods. J. Agric. Food Chem. 33: 528-530.

251. Newsome, W.H. (1986). Development of an enzyme-linked immunosorbent assay for triadimefon in foods. Bull. Environ. Contam. Toxicol. 36: 9-14.

252. Newsome, W.H. Determination of iprodione in foods by ELISA. In: Pesticide Science and Biotechnology, R. Greenhalgh and T.R. Roberts eds., Blackwell Scientific, Ottawa pp. 349-352. 1987.

253. Jung, F., Meyer, H.H.D. and Hamm, R.T. (1989). Development of a sensitive enzyme-linked immunosorbent assay for the fungicide fenpropimorph. J. Agric. Food Chem. 37: 1183-1187.

254. Meyer, H.H.D. Possible ways of improving enzymeimmunoassay (EIA) techniques and their application in animal production. In: Nuclear and Related Techniques in Animal Production and Health; ed. Food and Agricultural Organization of the UN; Int. Atomic Agency Vienna; pp. 255-262 1986.

255. Meyer, H.H.D. and Guven, B. (1986). Improvement of micrtitration plate enzyme immunoassays for steroid determination n by a second antibody technique. J. Steroid Biochem., Suppl. 25: 139.

256. Kitagawa, T., Kawasaki, T. and Munecika, H. (1982). Enzyme immunoassay of blasticidin S with high sensitivity: a new and convenient method for preparation of immunogenic (hapten-protein) conjugates. J. Biochem. 92: 585-590.

257. Chung, S.Y., Johnsen, P.B. and Klesius, P.H. (1990). Development of an ELISA using polyclonal antibodies specific for 2-methylisober nol. J. Agric. Food Chem. 38: 410-415.

258. Wie, S.I. and Hammock, B.D. (1982). The use of enzyme linked immunosorbent assays (ELISA) for the determination of Triton X nonionic detergents. Anal. Biochem. 125: 168-176.

259. Ekins, R.P., Chu, F.W. and Biggart, E.M. (1990). Multianalyte immunoassay: the immunological "compact disk" of the future. J. Clin. Immunoassay 13: 169-181.

260. Richardson, M.L. and Bowron, J.M. (1985). The fate of pharmaceutical chemicals in the aquatic environment. J. Pharm. Pharmacol. 37: 1-12.

261. Aherne, G.W., English, J. and Marks, V. (1985). The role of immunoassay in the analysis of microcontaminants in water samples. Ecotoxicol. Environ. Safety 9: 79-83.

262. Arnold, D. and Somogyi, A. (1985). Trace analysis of chloramphenicol residues in eggs, milk, and meat: comparison of gas chromatography and radioimmunoassay. J. Assoc. Anal. Chem. 68: 984-990.

263. Rohner, P., Schallibaum, M. and Nicolet, J. (1985). Detection of penicillin G and its benzylpenicilloyl (BPO) - derivatives in cow milk and serum by means of an ELISA. J. Food Protect. 48: 59-62.

264. Brimfield, A.A., Hunter, K.W., Lenz, D.E., Benschop, H.P., Van Dijk, C. and De Jong, L.P.A. (1985) Structural and stereochemical specificity of mouse monoclonal antibodies to the organophosphorous cholinesterase inhibitor soman. Molec. Pharmacol. 28: 32-39.

265. Van de Water, C. and Haagsma, N. (1991). Analysis of chloramphenicol residues in swine tissues and milk: comparative study using different screening and quantitative methods. J. Chromatogr. 566: 173-185.

266. Van de Water, C. and Haagsma, N. (1990). Sensitive streptavidin-biotin enzyme-linked immunosorbent assay for rapid screening of chloramphenicol residues in swine muscle tissue. J. Assoc. Off. Anal. Chem. 73: 534-540.

267. Morgan, M.R.A., McNerney, R. and Chan, H.W.S. (1983). Enzyme-linked immunosorbent assay of ochratoxin A in barley. J. Assoc. Off. Anal. Chem. 66: 1481-1484.

268. Ekins, R., Chu, F. and Biggart, E. (1989). Development of microspot multi-analyte ratiometric immunoassay using dual fluor ascent-labelled antibodies. Anal. Chim. Acta 227: 73-96.

269. Duchatel, J.P. and Maghuin-Rogister, G. (1985). Free and conjugated zeranol residues determined by radioimmunoassay in urine and plasma of calves treated with forplix. Ann. Rech. Vet. 16: 93-97.

270. Warner, R., Ram, B.P., Hart, P. and Pestka, J.J. (1986). Screening for Zearalenone in corn by competitive direct enzyme-linked immunosorbent assay. J. Agric. Food Chem. 34: 714-717.

271. Teshima, R., Kawase, M., Tanaka, T., Hirai, K., Sato, M., Sawada, J., Ikebuchi, H., Ichinoe, M. and Terao, T. (1990). Production and characterization of a specific monoclonal antibody against mycotoxin zearalenone. J. Agric. Food Chem. 38: 1618-1622.

272. Liu, M.-T., Ram, B.P., Hart, P. and Pestka, J.J. (1985). Indirect enzyme linked immunosorbent assay for the mycotoxin Zearalenone. Appl. Environ. Microbiol. 50: 332-336.

273. Stanbro, W.D., Newman, A.L. and Hunter, K.W. Applying biotechnology and microelectronics for environmental analysis. In: Biotechnology for Crop Protection. Ed. P.A. Hedin, J.J. Menn, R.M. Hollingworth; ACS Symposium Series 379, pp. 331-337 1989.

274. Wylie, D.E., Carlson, L.D., Carlson, R., Wagner, F.W. and Schuster, S.M. (1991). Detection of mercuric ions in water by ELISA with a mercury-specific antibody. Anal. Biochem. 194: 381-387.

275. Chait, E.M. and Ebersole, R.C. (1981). Clinical analysis: a perspective on chromatographic and immunoassay technology. Anal. Chem. 53: 682A-692A.

276. Al-Rubae, A.Y. The enzyme-linked immunosorbent assay, a new method for the analysis of pesticide residues". Ph.D. Thesis, Pennsylvania State University 1978.

277. Goh, K.S., Hernandez, J., Powell, S.J. and Greene, C.D. (1990). Atrazine soil residue analysis by enzyme immunoassay: solvent effect and extraction efficiency. Bull. Environ. Contam. Toxicol. 45: 208-214.

278. Goh, K.S., Hernandez, J., Powell, S.J., Garretson, C., Troiano, J., Ray, M. and Greene, C.D. (1991). Enzyme immunoassay for the determination of atrazine residues in soil. Bull. Environ. Contam. Toxicol. 46: 30-36.

279. Thurman, E.M., Meyer, M., Pomes, M., Perry, C.A. and Schwab, A.P. (1990). Enzyme-linked immunosorbent assay compared with gas chromatography/mass spectrometry for the determination of triazine herbicides in water. Anal. Chem. 62: 2043-2048.

280. Stocklein, W., Gebbert, A. and Schmidt, R.D. Binding of triazine herbicides to antibodies in anhydrous organic solvents Anal. Letters 23, 1465-1476, 1990.

281. Weetal, H.H. (1991). Antibodies in water immiscible solvents, immobilised antibodies in hexane. J. Immunol. Methods 136: 139-142.

282. Hock, B. (1991). Enzyme immunoassays for the determination of s-triazines in water samples: two interlaboratory tests. Anal. Letters 24: 529-549.

283. Harrison, R.O., Braun, A.L., Gee, S.J., O'Brien, D.J. and Hammock, B.D. (1989). Evaluation of an enzyme-linked immunosorbent assay (ELISA) for the direct analysis of molinate (Odram) in rice field water. Food and Agric. Immunol. 1: 37-51.

284. Ruitenberg, E.J., van Amstel, J.A., Brosi, B.J.M. and Steerenberg, P.A. (1977). Mechanization of the enzyme-linked immunosorbent assay (ELISA) for large scale screening of sera. J. Immunol. Methods 16: 351-359.

285. Ng, R.H. (1991). Immunoassay automation. J. Clin. Immunoassay 14: 59-60.

286. Dudley, R.F. (1991). The Ciba Corning ACS: 180 automated immunoassay system. J. Clin. Immunoassay 14: 77-82.

287. Jefferson, R. (1991). The Beckton Dickinson affinity immunoassay system. J. Clin. Immunoassay 14: 89-93.

288. Van Ginkel, L.A. (1991). Immunoaffinity chromatography, its applicability and limitations in multiresidu e analysis of anabolizing doping agents. J. Chromatogr. 564: 363-384.

289. Ohmicron, personal communication. 1991.

289. Environmental Diagnostics, Inc. Annual Report, 1989.

290. Walters, R.R. (1985). Affinity chromatography. Anal. Chem. 57: 1099A-1114A.

291. Glencross, R.G., Abeywardene, S.A., Corney, S.J. and Morris, H.S. (1981). The use of oestradiol-17b antiserum covalently coupled to Sepharose to extract oestradiol-17b from biological fluids. J. Chromatogr. 223: 193-197.

292. Hamers, A.R.M. and 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, Waginen, Netherlands, 30 May 1990, pp.63-70, 1990.

293. Davis, G.C., Hein, M.B. and Chapman, D.A. (1986). Evaluation of immunosorbents for the analysis of small molecules. J. Chromatog. 366: 171-189.

294. Van de Water, C. and Haagsma, N. (1987). Determination of chloramphenicol in swine muscle tissue using a monoclonal anti body-mediated clean-up procedure. J. Chromatogr. 411: 415-421.

295. Mortimer, D.N., Gilbert, J. and Shepherd, M.J. (1987). Rapid and highly sensitive analysis of aflatoxin M1 in liquid and powdered milks using an affinity column cleanup. J. Chromatog. 407: 393-398.

296. Van de Water, C., Tebbal, D. and Haagsma, N. (1989). Monoclonal antibody mediated clean-up procedure for the high performance liquid chromatographic analysis of chloramphenicol in milk and eggs. J. Chromatog. 478: 205-215.

297. Van Ginkel, L.A., Stephany, R.W., Van Rossum, H.J., Steinbuch, H.M., Zomer, G., Van De Heeft, E. and De Jong, A.P.J.M. (1989). Multi-immunoaffinity chromatography: a simple and highly selective clean-up method for multi-anabolic residue analysis of meat. J. Chromatogr. 489: 111-120.

298. Rang, K.T., XU, Q.H., Shao, R.X. and Gong, X.Q. (1986). Radioimmunoassay for paraoxon. Acta. Pharmacol. Sin. 1: 93-96.

299. Huber, S.J. and Hock, B. Atrazine in water. In: Methods of Enzymatic Analysis. Eds.: J. Bergmeyer and M. Grabl, VCH Verlagsgesellschaft; pp.438-451 1986.

300. Niewola, Z., Benner, J.P. and Swaine, H. (1986). Determination of paraquat residues in soil by an enzyme linked immunosorbent assay. Analyst 111: 399-403.

301. Li, Q.X., Gee, S.J., McChesney, M.M., Hammock, B.D. and Seiber, J.N. (1989). Comparison of an enzyme-linked immunosorbent assay and a gas chromatographic procedure for the determination of molinate residues. Anal. Chem. 61: 819-823.

302. Kuniyuki, A.H. and McCarthy, S. A novel method for the development of monoclonal antibodies to Surflan. Presented at Sixth International Congress of Pesticide Chemistry, Poster Session, Ottawa, August 11-15 1986 and cited in 48.

303. Monroe, D. (1984). Enzyme immunoassay. Anal. Chem. 56: 920A-931A.

304. Baselt, R.C. (1989). Inappropriate use of immunoassays as a quantitative tool. J. Anal. Toxicol. 13: 1.

305. Schultz, J.S. (1991). Biosensors. Sci. Amer. Aug.: 64-69.

306. Afghan, B.K., Carron, J., Goulden, P.D., Lawrence, J., Leger, D., Onuska, F., Sherry, J. and Wilkinson, R.J. (1987). Recent advances in ultratrace analysis of dioxins and related halogenated hydrocarbons. Can. J. Chem. 65: 1086-1097.

307. Tomita, M., Suzuki, K., Shikmosato, K., Kohama, A. and Ijiri, I. (1988). An enzyme-linked immunosorbent assay for plasma paraquat levels of poisoned patients. Forensic Science International 37: 11-18.

308. Morgan, M.R.A., Kang, A.S. and Chan, H.W.S. (1986). Aflatoxin determination in peanut butter by enzyme-linked immunosorbent assay. J. Sci. Food and Agric. 37: 908-914.

309. Groopman, J.D., Trudel, L.J., Donahue, P.R., Marshak-Rothstein, A. and Wogan, G.N. (1984) High affinity monoclonal antibodies for aflatoxins and their application to solid-phase immunoassays. Proc. Natl. Acad. Sci. USA 81: 7728-7731.

310. Mortimer, D.N., Shepherd, M.J., Gilbert, J. and Morgan, M.R.A. (1987). A survey of the occurrence of aflatoxin B1 in peanut butters by enzyme-linked immunosorbent assay. Food Additives and Comtam. 5: 127-132.

311. Anon ImmunoTechnology Catalogue and Handbook. Pierce, Rockford, Il, USA, 1990.

312. Bushway, R.J., Perkins, B., Savage, S.A., Lekousi, S.L. and Ferguson, B.S. (1989). Determination of atrazine residues in food by enzyme immunoassay. Bull. Contam. Toxicol. 42: 899-904.

313. Harrison, R.O. and Nelson, J.O. (1990). Analysis of maleic hydrazide in potatoes by competitive inhibition enzyme-linked immunosorbent assay. Food Chemistry 38: 221-233.

314. Ekins, R.P. The precision profile: its use in assay design, assessment and quality control. In: Immunoassays for Clinical Chemistry, Eds. W.M. Hunter and J.E.T. Corrie; Churchill Livingstone Edinburgh 76-105. 1983.

315. Hlady, V., Lin, J.N. and Andrade, J.D. (1990). Spatially resolved detection of antibody-antigen reaction on solid/liquid interface using total internal reflection excited antigen fluorescence and charged coupled device detection. Biosensors 5: ??.

Table 1. Advantages and limitations of IA techniques

ADVANTAGES	LIMITATIONS
Wide applicability (43, 48, 50, 58, 275)	High development costs (58, 69, 218, 275)
Complement GC or LC methods (31, 42, 44, 48, 58, 193)	Haptens can be difficult to synthesize (52)
Sensitive (36, 40, 42, 43, 58, 68, 69, 80, 275)	Limited availability of antisera (34, 50, 52, 124)
Specific (36, 42, 43, 44, 57, 65, 66)	Can be vulnerable to cross-reacting compounds and non-specific interferences (52, 66, 68, 83)
Cost effective analysis of small volume samples (42, 44, 45, 52, 66, 68, 124)	Requires independent confirmation (52, 77, 305)
Rapid with high sample throughput (42, 52, 66, 68, 69, 80, 124, 216, 304)	Inappropriate for small sample loads (44) or multi-residue determinations (52, 51, 66)
Ease of use (124, 125)	Lack of acceptance, conservative attitudes (44, 50, 69)
Usually reduced sample preparation (42, 46, 66, 124, 125, 193, 216, 218, 275)	
Simultaneous analysis of multiple samples (42)	
Ideal for large sample loads; easily automated (31, 34, 42, 44, 45, 46, 50, 51, 52, 66, 68)	
Suited to field use (31, 44, 48, 77)	

Table 2: 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	39
PCBs	2-NH_2-2',4,4',5,5'-PCBP	Convert to amide using succinic anhydride; couple to HSA using EDC[1]	154
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	156, 157
PCDDs	1-NH_2-3,7,8-TCDD	Convert to adipamide; couple using Mixed Anhydride Path; BSA/RSA/TYG	37, 146,147
PCDDs	2-NH_2-3,7,8-TCDD	Convert to adipamide; couple using Mixed Anhydride Path; BSA; TYG	145
2,3,7,8-TCDF	4-NH_2-2,7,8-TCDF	Convert to adipamide; couple using Mixed Anhydride Path; BSA/TYG	38
Benzo-a-pyrene	6-NH_2benzo-a-pyrene	BSA	158
2,4-D	2,4,-Cl_2-5-NH_2-D	Convert to R-N_2^+ and couple directly to BSA (Diazonium Path)	160
2,4-D	-COOH	Mixed anhydride method; HSA, BSA, HGG, BGG	162
2,4-D Picloram	-COOH -COOH	Convert to active ester using NHS[2] and DCC[3] and couple to protein (Active Ester Path); BSA	164, 205
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	161
Atrazine	R-NH$(CH_2)_5$COOH	Prepare amino caproic acid derivative from cyanuric acid; conjugate via Mixed Anhydride Path; BSA/RSA	165, 167
Atrazine	R-$SOCH_3$	Couple directly to sulfhydryl and lysine-NH_2 groups of haemocyanin	166, 300

Atraxine and simazine	At(6)-$NH(CH_2)_5COOH$	raised in sheep	177
Cyanazine	R(4)-NH-$(CH_2)_5CONH$Prot.		61, 170
Terbutryn	R-SOCH3	Couple directly to sulfhydryl and lysine-NH_2 groups of BSA	172
Atrazine	R-Cl	"Derivatize and conjugate to BGG using a modified CDI procedure"	179
Atrazine and hydroxyatrazine	R(4)-$NH(CH_2)_4COOH$	NHS Active Ester Path; BSA/KLH	181
Atrazine and Simazine	a:R(4)$NH(CH_2)_nCOOH$ b:R(2)$S(CH_2)_2COOH$	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	183
Atrazine and propazine	R-$NH(CH_2)_5COOH$	CDI using (a) CMC (b) DCC (c) EDC; BSA	168
s-Triazines	(a) $RSOCH_3$ (b) R-Cl	Couple directly to free NH_2 groups; BSA	182
Bentazon	R-N-$CH_2C_6H_4CH_2COOH$	NHS active ester pathway;BSA/KLH	208
Imazamethabenz	R-COOH	Water soluble CDI path using EDC; HSA	207
Aminotriole	-SH, -OR, -COOH	Library of 13 antigens prepared using 7 haptens and variety of spacer groups; hapten's NH_2 group was protected	208
Trifluralin	-C_2H_4COOH	Synthesise propionic acid trifluralin; conjugate using mixed anhydride method	209
Norflurazon	-C_4H_8COOH	Synthesise butyric acid derivative of 4-NH_2-Norflurazon; conjugate to BSA & RSA using mixed anhydride procedure	210
Deethylatrazine and deisopropylatrazine	-$(CH_2)_5COOH$	Couple to BSA using CMC	175
Permethrin	Phenothrin-COOH	Mixed anhydride method; KLH and BSA	214
Cypermethrin	PBA-COOH CYP-COOH	CDI method	61
Aldicarb	-C(O)Cl	Convert to aldicarb oxime chloroformate; couple to trans-4-(amino-methyl) cyclohexane carboxylic acid and then conjugate to BSA using mixed anhydride path	240
Paraquat	R-COOH	Prepare hexanoic acid derivative of monoquat; couple to BSA/KLH using CDI	186, 188, 189, 192
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	187

Paraquat	R-COOH	Prepare valeric acid derivative; convert to mixed anhydride using isobutyl chlorocarbonate; couple to KLH; OA; HSA; BSA; CONA	193, 194
Diclofop-methyl	D-COOH	Convert ester to acid; couple to NH_2 using CMC[4] (Water Soluble CDI); BSA	196
Clomazone	Amino-Clomazone	Diazo linkage	197
Clomazone	p-NH_2-Clomazone	Diazo linkage; BSA	198
Alachlor	R-Cl	Thiolate proteins and couple directly to hapten	199
Metolachlor	Ar-O$(CH_2)_4$COOH	NHS Active Ester Method; BSA/KLH	200
Metazachlor	R-Cl	Thiolate proteins and couple directly to hapten	201
Maleic hydrazide	(a) R-COOH	(a) NHS Active Ester Path; KLH	202
Chlorosulfuron	D-C_6H_4-NH_2	Diazonium Path; KLH; BSA	67
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	204
Aldrin (Dieldrin)	Analog-COOH	Active Ester Path; HSA	35
Chlordane		Derivatize at C-2 and couple to BGG using CDI	211
Endosulfan	D-$(OH)_2$	Convert diol to hemisuccinate using succinic anhydride; NHS Active Ester Path; KLH	212, 213
S-Bioallethrin	D-OH	Convert to ester using succinic anhydride; Active Ester Path; BSA/KLH	215
Diflubenzuron and BAY SIR	R-COOH	(a) NHS Active Ester Path; (b) Water soluble carbodiimide path using CMC[4]; BSA; KLH; OVA	222, 223,
Parathion	R-NH_2	Diazonium Path	34, 225
Parathion	(i) 2-amino parathion (ii) reduced parathion	Several strategies evaluated: (a) Diazo linkage; (b) couple (i) and (ii) using gluteric di-aldehyde; (c) convert (i) to amide using succinic anhydride and then use Mixed Anhydride Path; BSA; RSA	226
Paraoxon	R-NH_2	Diazonium Path; KLH/BSA	228, 230, 233
Paraoxon	(1) R-P-Cl (2) R-P-$NHCH_2CH_2COOH$	(1) conjugate directly to BSA (2) NHS Active Ester Path; BSA	231

Soman	R-(C_6H_9)-NH_2	Diazonium Path; BSA	234, 235
MATP	NH_2	Diazonium Path	236, 237
Organophosphates	(a) DCP-COOH (b) TPB-COOH	Water soluble CDI using EDC and dioxane to improve solubility; BSA	232
DDA	R-COOH	Convert to acid chloride using $SOCl_2$; couple to protein via amide bond; Bovine fibrinogen	33
DDA	R-COOH	React DDA anhydride with BSA/RSA	239
DDT	R-COOH	Convert to anhydride and conjugate to NH_2; BSA	32
Malathion	D-(CO_2)O	Couple th4e anhydride to BSA	32
Malathion	D-COOH	Convert to acid chloride using $SOCl_2$; couple to protein using amide bond; Bovine fibrinogen	33
2-Aminobenzimidazole	R-NH_2	React with thiophosgene to form the isothiocyanate; couple directly to NH_2; OVA	36
Benomyl, methyl 2-benzimidazole-carbamate, thiabendazole	R-NH_2	Convert to amide using succinic anhydride; Couple to NH_2 using water soluble EDC[1] (Water soluble CDI Path); BSA	247, 248
Metalaxyl	R-COOH	Water Soluble CDI Path; HSA	250
Triadimefon	RCO; Reduce C=O to C-OH	Convert to ester using succinic anhydride; couple to HSA using Water Soluble CDI	251
Iprodione	R-COOH	Water Soluble CDI Path; HSA	252
Fenpropimorph	R-COOH	NHS Active Ester Path; BSA	253
Ochratoxin A	-COOH	Convert to mixed anhydride using isobutyl chlorocarbonate; couple to BSA	267
Triton-X detergents	OH	Convert to ester using succinic anhydride; Mixed Anhydride Path; KLH; BSA	258
Blasticidin S	BLS-NH_2	Acylate BLS using MBS; use sodium borohydride to reduce disulfide bonds in BSA; conjugate MBS-hapten to reduced protein	256
Surflan	-NH_2	Activate gel using NHS; couple to hapten's sulfonamide group group; Affigel 10	303

1: 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide
2: N-hydroxysuccinimide
3 dicyclohexylcarbodiimide
4: 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-4-toluene sulfonate

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

Analyte	ABs	Format	Label	Sep. Sys.	Range(dl) Buffer	Range(dl) Matrix	Reference
PCDDs 2,3,7,8-TCDD	P	RIA	1-N-[5-^{125}I-valeramido]-3,7,8-TCDD	DAB[3]	F12[1] 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)	37, 137
PCDDs 2,3,7,8-TCDD	M	RIA	[^{125}I]-second AB	SP	Inadequate competition		145
PCDDs	P	RIA	1-N-[5-^{125}I-valeramido]-3,7,8-TCDD	DAB	12.5 pg-1 ng (27 pg)	Fish: (67 ppt)	140, 141
PCDDs 2,3,7,8-TCDD	M	EIA	Enzyme labelled second AB	IH[5]	100 pg - 10 ng (<500 pg) I_{50}: 1 - 2.5 ng		146, 147
PCDDs	M	EIA	Enzyme labelled 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)	51, 97
PCDDs 2,3,7,8-TCDD	p	RIA	^{3}H-labelled 2,3,7,8-TCDD (40 Ci/mmol)	DCC	(a) 20 pg - 2 ng (21 pg) I_{50}: 350 pg (b) 2.5 pg - 200 pg (3.9 pg); I_{50}: 42 pg		144
PCDFs	P	RIA	4-N-[5-^{125}I-valeramido)-2,7,8-TCDF	DAB	20 pg - 4 ng (20 pg) I_{50}: 150 pg		38
PCBs 4-MCBP 453'4'TCBP 262'6'TCBP	P	RIA	^{125}I-Iodovaleramido-BP	DAB	2-NH_2 -3'4'45 TCBP: 0.5-100 ng (0.5ng)[2], I_{50}: 5 ng; (dl 343'4'TCBP/2,6,2',6'TCBP: 1.0ng)	25-450 ng Aroclor 1254; 5-300 ng other BPs & Aroclors	39
PCBs 22'44'55'HCBP	P	RIA	2-[^{125}I-Iodo]-2'44'55'-HCBP	DCC[4]	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)	154
PCBs Arocolor 1248		EIA			(8.4 ng/well)	17 ppb soil extract (concentrated 50X)	155
PCP	M	Probe	Capacitative affinity sensor				273
PCP	M	EIA	Enzyme labelled 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)	159

4-acetamidobiphenyl	P	RIA	[^{125}I]-tyramine derivative (16 Ci/mmol)	DAB	66 pg - 40 ng (5.7 pg) I_{50}: 1 ng		156
N,N'-diacetylbenzidine	P	RIA	[^{125}I]-tyramine derivative	DAB	8.3 pg - 530 pg (6.2 pg) I_{50}: 100 pg		157
Benzo-a-pyrene	M	EIA	Enzyme labelled second AB	IH	$3x10^2$ fmol - 10^4 fmol ($3x10^2$ fmol)^2; I_{50}: 4 pmol	Urine: I_{50}: 12-15 pmol	158

1: Serum pool.
2: Estimated from lower limit of calibration curve.
3: Double AB method.
4: Dextran coated charcoal.
5: Immobilized hapten.

Table 4:

IAS FOR HERBICIDES

Analyte	ABs	Format	Label	Sep. Sys.	Range (dl) Buffer	Range (dl) Matrix	Reference
(1) 2,4-D & (2) 2,4,5-T	P	RIA	[^{125}I]-hydroxyl phenyl diazene derivative;	PA[1]	0.1 - 100 ng (200 pg)[5] (1) I_{50}: 6.4 ng/100µL (2) I_{50}: 1.4 ng/100µL	(1) (100 ppt) (river water)	160
2,4-D	P	RIA	(a) [^{125}I]-tyramine derivative (b) Tritiated 2,4-D (12.6 Ci/mmol)	PEG	(a) did not compete (b) I_{50}: 4.5 pM	In urine: 0.1 - 100 ng (100 pg); I_{50}: 1 ng	162, 163
2,4-D	P	EIA	Labelled 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)	161
2,4-D	P	EIA; RIA	(a) labelled DAB (b) [^{3}H]glycine-hapten	IH $(NH_4)_2SO_4$	(a) 100 ng/mL - 10 µg/mL (100 ng/mL)[5]; I_{50}: 2 µg/mL (b) 50 ng/mL - 10 µg/mL (50 ng/mL)[5]; I_{50}: 1 µg/mL	In river water & urine: (a) 100 ppb - 10 ppm (b) 50 ppb - 10 ppm	164
Triazines (Atrazine)	P	EIA; RIA	(a) Biotinylated DAB/streptavidin peroxidase (b) [^{125}I]-tyrosine derivative	IH	(100 pg/mL) (1 ng)		165, 167
Atrazine	P	EIA	Labelled hapten	IAB[2]	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.		166, 300
				IAs[3]	IgG: 11 pg/mL - 55 ng/mL (11 pg/mL); I_{50}: 21.5 ng/20mL. Affinity purified ABs: 110 fg/mL - 550 pg/mL (100 fg/mL); I_{50}: 108 pg/20 mL		
Atrazine	P	EIA	Labelled hapten		(20 pg/ml)		174
Terbutryn	P	EIA; RIA	(a) enzyme labelled DAB	IAB	4.8 - 290 ng/assay (4.8 ng); I_{50}: 69 ng/assay	River water: 25 - 1500 ppb (25 ppb)	172, 173
				IAs	1.9 ng - 14.4 µg (1.9 ng)[5]	0.1 - 600 ppb (0.1 ppb)	
			(b) [^{14}C]Terbutryn	SP	Similar to EIA		

Cyanazine	P	EIA		IH		(0.5 ppb in water; 0.005 ppb wi8th SPE; 0.01 ppm in soil)	61, 170
Atrazine	P	EIA	Labelled hapten	IAt[4]	0.5 - 10 ng/mL (0.1 ng/mL) (50 pg - 1 ng/tube); I_{50}: 0.4 ng/mL		179; Ferguson, personal com-munication
				IAB	30 pg/mL - 5 ng/mL (20pg/mL); I_{50}: 250 pg/mL		
Atrazine/simazine	P	FIA	labelled hapten	IAB	0.01 - 1.0 ng/mL		177
(1)Atrazine & (2)-hydroxy atrazine	M	EIA	Labelled 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)	181
Triazines	P	EIA	Labelled 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 (0.3ppb-1ppm)	183, 184
(a) Deethylatrazine (b) deisopropylatrazine	P	EIA	Labelled 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		175
Atrazine / propazine	P	EIA	Labelled 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		168
s-Triazines	M	EIA	Labelled 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		182
Aminotriole	P	EIA	Labelled DAB	IH	(0.02 µM - 50 µM(0.02 µM)		206
Paraquat	P	RIA			50 - 1600 ng/mL (50 ng/mL); I_{50}: 100 ng/mL		185
Paraquat	P	RIA		C	0.5 - 10 ng (0.6 ng/mL: niewola et al. 1985 (189) cite a DL of 6 ng/mL for the RIA) Sensitivity of optimized assay =120 pg/mL)	In serum: (0.6 ng/tube)	186

Paraquat	P	RIA	(a) [^{3}H]Paraquat (1.35 Ci/mmol)	SP	(a) 1 - 100 ng/mL (0.8 ng/mL); I_{50}: 10 ng/mL	(a) In serum: 10 - 250 ppb (3.6 ppb)	187
			(b)[^{125}I]-phenol derivative (130 Ci/mmol)	SP	(b) 0.29 - 28.6 ng/mL (0.1 n-g/mL)	(b) (0.25 ppb in serum)	
Paraquat	P	EIA	Labelled DAB	IH	(0.3 - 10 ng/mL); I_{50}: 8x10^{-7}M		188
Paraquat	M& P	EIA	Labelled DAB	IH	(M): 0.8 - 12 ng/mL (0.5 ng/m-L); I_{50}: 3 ng/mL (P): 0.08 - 10 ng/mL (0.08 ng/-mL)[5]; I_{50}: 2 ng/mL	In serum: 0.8 - 50 ppb (0.5 ppb); In soil (200 ppb)	189, 190, 301
Paraquat	P	EIA	Labelled DAB	IH	0.1 - 27 ng/mL; I_{50}: 2 ng/mL	Variety of ma-trices: (0.1 - >100 ppb); milk: (<1ppb) I_{50}: 0.3 ppb	194, 195
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)		192
Diclofop-methyl	P	EIA; FIA	(a) Enzyme labelled hapten (b) Fluorescein labelled hapten	DAB-+PEG PEG	(a) 10 - 75 ng/mL (23 ng/mL); I_{50}: 27 ng/mL (b) 20 - 200 ng/mL (45 ng/mL; 9 ng/tube); I_{50}: 32 ng/mL	(a) soil: (.023 ppm); milk: (.23 ppm); urine: (.115 ppm) (b) soil: (.045 ppm); milk: (.45 ppm); urine: (.225 ppm)	196
Clomazone	P	EIA	Labelled DAB	IH	0.5 - 500 ng/mL (0.5 ng/mL)[5]; I_{50}: 12 ng/mL	Soil extracts: 0.01 - 10 ppm	197
Clomazone	P	EIA	Labelled DAB	IH	1.37-250 (ng/mL) (1ng/mL); I_{50}: 25 ng/mL	Soil extracts (5 ppb)	198
Alachlor	P	EIA	Labelled DAB	IH	Deionised H_2O: 0.2 - 8 ng/mL (0.2 ng/mL)[5]; I_{50}: 0.5 ng/mL		199
Metolachlor	M	EIA	(1) Labelled hapten (2) Labelled 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		200
Metazachlor	P	EIA	Labelled hapten	IABs	10 - 1000 pg/mL (10 pg/mL) I_{50}: 100 pg/mL		201
Maleic hydrazide	M	EIA	Labelled DAB	IH	10 ng/mL - 11 µg/mL (0.11 µg/-mL); I_{50}: 0.84 ppm	(1 ppm in potato extract)	202, 314
Chlorosulfuron	P	EIA	Labelled DAB	IH	I_{50}: 90 ng/mL	In soil: (0.4 - 1.2 ppb)	87
Imazamethabenz	P	EIA	Labelled DAB	IH	0.5 - 32 ng/mL I_{50}: 12.6 ng/mL	12.5 - 200 ppb in wheat & barley	207

Bentazon	P	EIA	Labelled DAB	IH	I_{50}: 110 µM		208
Molinate	P	EIA	Labelled 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)	204
Molinate	P	EIA	Labelled DAB	IH	10 - 500 ng/mL (21 ng/mL); I_{50}: 106 ng/mL		52, 283
Molinate	P	EIA	Labelled DAB	IDAB	I_{50}: 20 ng/mL		52
Molinate	P	EIA	Labelled DAB	IH	(15 ng/mL)	(10 ppb in water; 0.1 ppb in water extract; 30 - 60 ppb in soil extract)	42, 302
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 & urine: 50 -5000 ppb	164
Picloram	P& M	EIA	Labelled 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	205
Thiobencarb	P	EIA	Labelled DAB	IH	20 - 1000 ng/mL (1 ng/mL; practical 10 ng/mL); I_{50}: 100 ng/mL		42
Trifluralin	P	EIA	Labelled DAB	IH	(0.1 - 1 ppm)		209
Norfluazon	P	EIA	Labelled DAB	IH	1 - 10000 ng/mL (1 ng/mL); I_{50}: 250 ng/mL		210

1: Protein A; 2: AB immobilized on microtitre plates; 3: ABs immobilized on polystyrene beads; 4: ABs immobilized on tube walls; 5: estimated from lower limit of calibration curve by present author.

Table 5: IAS for INSECTICIDES

Analyte	ABs	Format	Label	Sep. Sys.	Range(dl) Buffer	Range (dl) Matrix	Reference
(1)Aldrin & (2)Dieldrin	P	RIA	[^{125}I]-tyramine derivative (3.3 Ci/mmol)	DAB	(1) 2 - 100 pmole (700 pg); I_{50}: 8 ng (2) 0.2 - 100 pmole (150 pg); I_{50}: 1.6 ng		35
Chlordane	P	EIA	Labelled hapten	IH	2.5 - 80 ng/tube (25 ng/mL)[1]; I_{50}: 110 ng/mL	Soil extracts: 0.8 - 3000 ppm	211
Endosulfan	P	EIA	Labelled hapten	IAB	3 - 400 ng/mL (3 ng/mL)[1]; I_{50}: 135 ng/mL		212
s-Bioallethrin	P	RIA	[^{3}H]-tyramine derivative	C	0.1 - 10 nmoles (0.1 nmoles); I_{50}: 1.2 nmoles		216
	P	EIA	Labelled 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	193, 217
(1) Diflubenzuron & (2) BAY SIR 8514	P	EIA	Labelled DAB	IH	(1) 8 - 200 ng/mL (8 ng/mL)[1]; I_{50}: 48 ng/mL (2) 10 - 400 ng/mL (10 ng/mL); I_{50}: 120 ng/mL	Water: (1 ppb) Milk: (40 ppb)	223
			Labelled 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, & (3)Penfluron	P	EIA	Labelled 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	224
Organophosphates	P	EIA	Labelled DAB	IH	I_{50}: 10^{-6} - 10^{-4} mol/L (I_{50}x5^{-1})		232
Parathion	P	RIA	[^{3}H] (300 mCi/mmol) or [^{14}C] (40 mCi/mmol) labelled hapten	DCC	4 - 400 ng (4 ng); I_{50}: 50 ng	Plasma & lettuce (10 -20 ng) = 0.1 ppm	225
Parathion	P	EIA	Labelled DAB	IH	(5 - 10 ng/mL); I_{50}: 58 ng/mL	0.025 - 0.05 ppm (fruit, veg., serum extracts)	276
Paraoxon	P	EIA	Labelled DAB	IH	10^{-10}M - 10^{-3}M (28 pg/mL); I_{50}: 8x10^{-6}M	Serum: (280 pg/-mL)	228, 229
Paraoxon	M	EIA	Labelled DAB	IH	10 - 100 µg/mL (1 µg/mL); I_{50}: 7x10^{-6}M		230

Paraoxon	P	RIA	[^{3}H]		100 pg - 3.2 ng (200 pg) I_{50}: 800 pg		299
Paraoxon	P	EIA	Labelled hapten	PT[2]	(10^{-6}M)	Water: (10^{-6}M)	233
Paraoxon	P	(1) RIA (2) EIA	[^{3}H]paraoxon [^{3}H]AChH	(1)$(NH_4)_2SO_4$ (2)homogenous	(1): 0.7 - 70 pmoles (300 pg) I_{50}: 7 pmoles (2): 10^{-7} - 10^{-6} M (10^{-7} M)		231
Soman	M	EIA	Labelled DAB	IH	10^{-6}M - 10^{-3}M (200 ng/mL); I_{50}: $8x10^{-3}$M		234
MATP (Soman-/OP Analog)	P	EIA	Labelled DAB	IH	10^{-8} - 10^{-4} mol/L (10^{-10} mol/L) I_{50}: 10^{-6}mol/L		236
MATP (Soman-/OP Analog)	M	EIA	Labelled hapten	IAB	10^{-7} - 10^{-4} mol/L ($1.3x10^{-7}$ mol/L; 975 pg/assay or 39 ng/mL)	Various matrices: ($2.1x10^{-7}$ - $4.9x10^{-6}$ mol/L)	237
Soman	M	EIA	Labelled hapten	IAB	10^{-7}-10^{-4} mol/L ($5x10^{-7}$ mol/L; 100 ng/mL)	Various matrices: ($1.3x10^{-6}$ - $2.0x10^{-6}$ mol/L)	238
Cypermethrin (PBA)	M	EIA			(50 ppb)		61
Permethrin	M	EIA	Labelled DAB	IH	0.4 ng - 20 ng (0.4 ng)[1] I_{50}: 1.55 ng	50 -500 ppb in beef	214
DDA	P	EIA	Labelled DAB	IH	10 - 100 ng/mL (10 ng/mL)[1]; I_{50}: 72 ng/mL	Urine: ng level	239
Aldicarb	P	EIA	Labelled hapten	IAB	15.6 - 2 µg (300 ng/mL) I_{50}: 125 ng	0.3 ppm stream water, 0.6 ppm plasma	241, 240
Bacillus thuringiensis sub sp. kurstaki toxin	P	EIA	Labelled DAB	IH	0.03 - 3 µg/mL (0.03 µg/mL)[1]; I_{50}: 6 µg/mL		242
Bacillus thuringiensis sub sp. kurstaki toxin	P	EIA	Labelled toxin	IAB	20 - 1000 ng/mL (20 ng/mL)[1]; I_{50}: 105 ng/mL		243
Bacillus thuringiensis sub sp. israelensis toxin	P	EIA	Labelled DAB	IH	15 - 1000 ng/mL (15ng/mL)[1]; I_{50}: 200 ng/mL	Water: no clean-up (100 - 1000 ppb); with SPE step (100 pg/mL)	244

1: Estimated from lower limit of calibration curve by present author.
2: Pinch test format.

Table 6: IAS FOR FUNGICIDES

Analyte	ABs	Format	Label	Sep. Sys.	Range(dl) Buffer	Range (dl) Matrix	Reference
2-Aminobenzinimidazole	P	FIA	Fluorescein labelled hapten	na	100 pg/mL - 160 ng/mL (100 pg/mL)		36
Benomyl & methyl 2-benzimidazolecarbamate	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	247, 248
Benomyl (1) & Thiabendazole (2)	P	EIA	Labelled 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))	248
MBC	P	EIA	Labelled hapten	IAB	1-26 ng/mL (100 - 2600 pg/tube)	(10 ppb juices; 30 ppb concentrates)	249
Metalaxyl	P	EIA	Labelled DAB	IH[1]	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)	250
Triadimefon	P	EIA	Labelled DAB	IH	1 - 16 ng/mL (1 ng/mL)[3];	Variety of produce 0.5 - 2 ppm	251
Iprodione	P	EIA	Labelled 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)	252
Fenpropimorph	P	EIA	Labelled hapten	IDAB[2]	20 pg/mL - 2 ng/mL (13 pg/mL); I_{50}: 300 pg/mL (6 pg/well)		253
Blasticidin S	P	EIA	Labelled hapten	DAB	100 pg/tube - 10 ng/tube (100 pg/tube); I_{50}: 1 ng/tube		256

1: Immobilized hapten
2: Immobilized double antibody technique
3: Estimated from lower limit of calibration curve by present author.

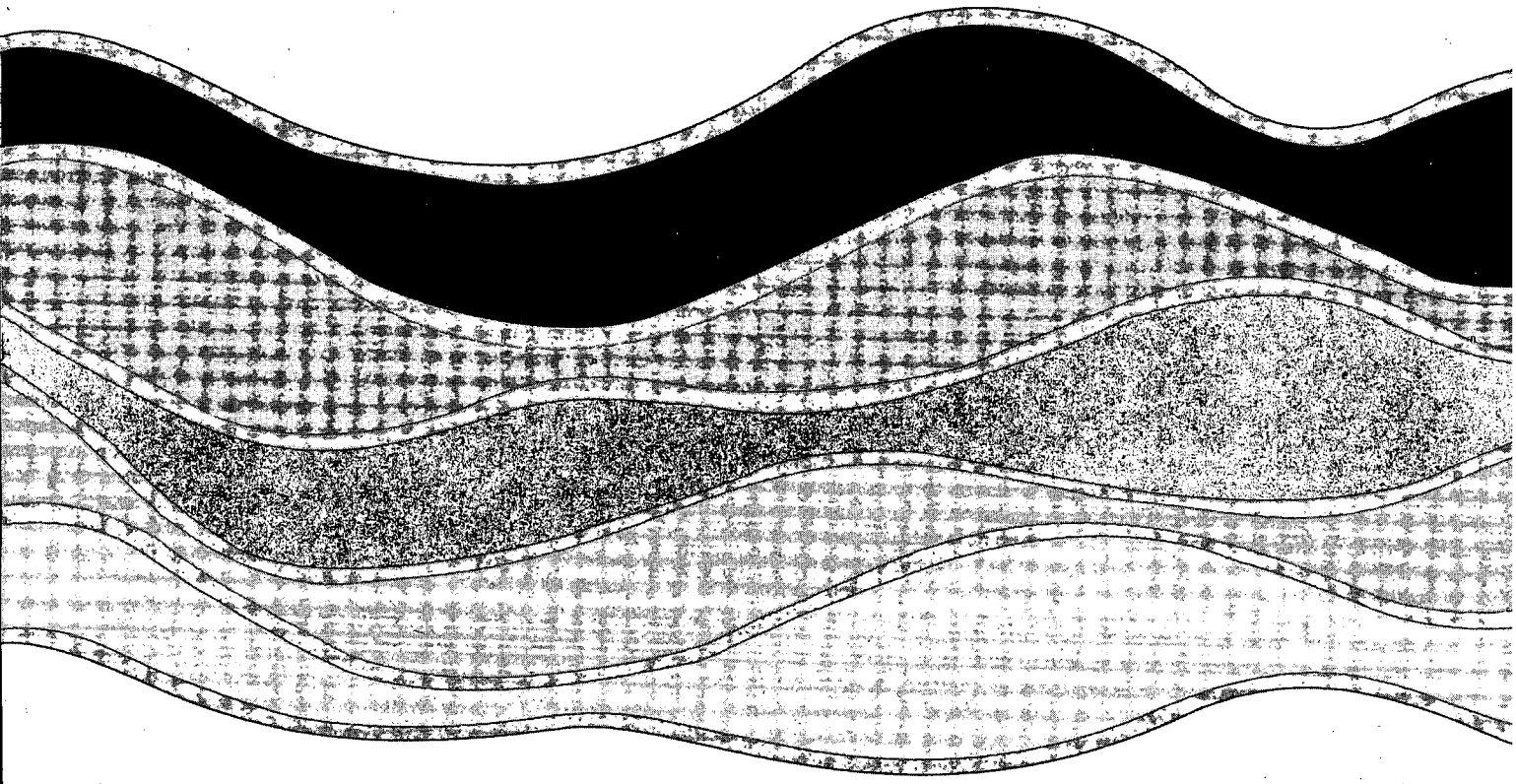
Table 7. Some examples of IAs that have been used to detect contaminants in aqueous matrices without clean-up.

Analyte	Matrix	Observations	Ref.
2,4-D	river water, urine, serum, plasma	sensitivity loss in plasma	162, 164
Picloram	river water, urine		164, 205
Molinate	water	ppm levels	204, 283, 302
Hydroxyatrazine, Atrazine	water		179, 181, 174, 184, 300, 168
Terbutryn	river water		172
Paraoxon	serum	slightly reduced sensitivity	228, 229
Parathion	serum	reduced sensitivity	225
Paraquat	plasma and urine		187, 308
Paraquat	milk		195
Fenpropimorph	tap water and soil percolation water		253
s-Bioallethrin	milk	reduced sensitivity	217
Diflubenzuron BAY SIR 8514	stagnant water		223
Alachlor	water	some matrix effects	199
Diclofop-methyl	serum and urine		196
PCP	groundwater		159
Deethylatrazine & deisopropylatrazine	water, groundwater	no matrixeffects	175
Paraoxon	blood		299
Soman	water, serum, milk	reduced sensitivity	238
Aldicarb	stream water		240
MBC	juicess	slight matrix effects	249

Table 8. Examples of IAs that have been used to detect contaminants in crude matrix extracts

Analyte	Matrix	Observations	Ref.
Benomyl	food, produce		247, 248
Metalaxyl	food, produce		250
Triadimefon	food, produce		251
Iprodione	food, produce		252
Chlordane	soil		211
Parathion	produce	reduced sensitivity	225
Picloram	soil		205
Hydroxyatrazine atrazine	soil		179, 181
Atrazine	soil	some positive interferences	277
Molinate	soil	positive interferences	302
Clomazone	soil	some negative interferences	197, 198
Paraquat Paraquat	soil beef, potatoes		190, 301 195
Chlorosulfuron	alkaline soil extracts	some matrix interferences	87
Diclofop-methyl	soil and biota		196
Metolachlor	soil	slight interference in direct EIA	200

Environment Canada Library, Burlington
3 9055 1017 7886 7



NATIONAL WATER RESEARCH INSTITUTE
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6

Environment Canada Environnement Canada

Canada

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX
C.P. 5050, BURLINGTON (ONTARIO) L7R 4A6

Think Recycling!

Pensez à recycler !