Monitoring human exposure to pesticides using immunoassay

Marja E. Koivunen[1,2], Shirley J. Gee[1], Mikaela Nichkova[1], Ki Chang Ahn[1], Bruce D. Hammock[1]

[1] Department of Entomology, University of California, Davis, CA 95616
[2] Current address: Antibodies Incorporated, P.O. Box 1560, Davis, CA 95617-1560

Immunoassays offer selective, sensitive and low-cost tools for the assessment of pesticide exposure through the measurement of parent compounds or key metabolites in biological fluids such as urine, blood or saliva. Recently conducted biomonitoring studies for paraquat and atrazine illustrate the strengths and weaknesses of the immunochemical approach. Development for improved assay throughput and sensitivity includes substitution of enzyme labels with fluorescent nanoparticle probes or luminescent acridinium labels together with the use of automated immunoanalyzers, immunosensors or microchips with flow-through systems.

Biological monitoring of exposure is currently applied in environmental and occupational toxicology as well as in epidemiological studies on the dose-response relationship between internal exposure and adverse health effects. All three types of biomarkers – exposure, effect and susceptibility – can be used for pesticide exposure assessment. However, the biomarkers most often used in pesticide studies, biomarkers of exposure, are the ones indicating recent or long-term exposure to a particular compound of interest (1) .

Chromatographic techniques used for exposure analysis (2,3) are often expensive requiring special instruments and extensive sample clean-up, extraction or derivatization. Modern immunochemical techniques offer simple, specific and sensitive tools for human exposure studies involving numerous samples in complex matrices (4).

Selection of Biomarkers and Sample Media

Biomarker

Overall, the choice of a biomarker for a particular compound requires extensive knowledge about its biotransformation and metabolism in humans. Studies on animals give only a partial answer to this question, and in some cases, a compound identified as a major metabolite in a high-dose animal study is not the same in humans exposed to concentrations relevant for occupational and nonoccupational settings (5). It has been suggested that in order to properly evaluate biomarkers of exposure to pesticides, human volunteers should be given low doses of the compound (*6*). Similarly, measurement of a parent compound or its main metabolites can rarely provide any information on the health risk unless it is tied into an epidemiological study with corresponding short and long-term health effect evaluations.

According to Hoet (7), biological monitoring of exposure to pesticides is aimed at the estimation of internal dose based on the fate of the compound in human body. Biological monitoring approaches can be categorized into four main types

1. Direct measurement of unchanged pesticides in biological matrices (2,4-D, pentachlorophenol, DDT, lindane, paraquat)
2. Determination of metabolites in biological matrices (atrazine mercapturate, 3-phenoxybenzoic acid (PBA), *cis*/*trans*–dichlorovinylcyclopropane acid (DCCA), 1-naphtol)
3. Quantification of biological effects related to internal dose (acetylcholinesterase activity)
4. Measurement of macromolecule adducts combined with target or non-target molecules (DNA and hemoglobin adducts)

Biological medium

The selection of biological medium for monitoring is determined based on the excretion pattern of the selected analyte as well as the ease and timing of sampling and the availability of data relating excretion to exposure. Depending

on the analyte, either xenobiotic parent compounds or metabolites can be analyzed in urine, which is by far the matrix most often used for biological monitoring of human exposure. Two obvious advantages of urine over blood are its ease of availability and the amount of sample available for analysis. In some cases, the concentration of toxicants or metabolites is higher in urine than in blood, which decreases the sensitivity requirement for the analysis method.

Blood is an excellent medium for biological monitoring but its sampling requires an invasive procedure which makes it less desirable for routine analysis in the field. For immunochemical methods, serum and plasma are preferred matrices over whole blood and ELISAs have been successfully applied in the analysis of human plasma for phenylurea (8) and triazine herbicides (9).The blood concentration of the parent compound is usually highest immediately following exposure, and the preferred time for sampling is easy to establish. However, the blood volume obtained is usually small, which means that ultrasensitive analytical techniques might be required. It should be kept in mind that an increase in sample size is usually accompanied by an increase in background noise. With immunochemical detection methods, this usually means that in order to remove the interfering substances from the sample matrix, sample preparation steps are needed.

Besides urine and blood, many other biological media are available for sampling. Hair, nails, saliva, milk, feces and fat tissue can be used for biomonitoring, but for all these matrices, tedious sample pretreatment and extraction methods are usually required prior to analysis. A few studies have used ELISA for the analysis of pesticide residue in saliva: Denovan et al. (10) analyzed atrazine in human saliva samples using ELISA, and concluded that the salivary concentration of atrazine was a good indicator for human exposure to this pesticide. The assay had a limit of detection of 0.22 ng mL^{-1} in saliva and could clearly distinguish among workers who had sprayed atrazine. Up to date, strong correlation with plasma and saliva concentrations has only been demonstrated in animal models for atrazine (11) and diazinon (12). More studies on the relationship between human plasma and saliva concentrations of environmental contaminants are needed in order to fully utilize saliva biomonitoring for estimation of absorption, metabolism and excretion.

Immunoassays for biomonitoring

In the past 10 years, the application of immunoanalytical techniques in human exposure studies has steadily increased. However, most field studies have been targeting human exposure to industrial chemicals and their metabolites (13-16). A review by Barr and Needham (3) listed only a few papers reporting use of immunochemistry in the analysis of pesticides or their metabolites in biological matrices. Since 2002, a few successful attempts have been made in our laboratory (17,18) to use these rapid, simple and cost-effective

methods for human biomonitoring of pesticide exposure. The recent rapid growth of immunoanalytical techniques in clinical chemistry can be attributed to the low cost and portability of assays – simple, yet sensitive immunoassays can be performed without extensive training, even in the field and in the point-of-care facilities. The increased availability of polyclonal, monoclonal and even recombinant antibodies for a variety of pesticides and their metabolites within the academia has contributed to the growth of immunochemical analysis in the field of human exposure monitoring. However, the availability of low-cost analysis kits and reagents still does not meet the increasing demand, which limits the application of immunoassays in the field studies. Another factor making immunoassays more applicable to human biomonitoring studies is the substitution of enzyme labels by fluorescent probes, which efficiently reduces background signals and enhances the limit of detection (LOD). Schobel et al. (19) have given a comprehensive review on immunoanalytical techniques and fluorescence detection suitable for pesticide residue monitoring in environmental and food analysis. Similarly, development of fluorescent probes and immunobiosensors is gradually advancing the technology from the conventional competitive microplate pesticide assays to a more efficient and sensitive detection of multiple analytes in complex biological matrices (20,21).

Special Requirements

Elimination of Interfering Substances

In biological samples, the interferences caused by matrix components can vary from sample to sample, and endogenous compounds with structural similarities with the target analyte can interfere with the immunochemical detection. In some cases, a simple sample dilution is enough to eliminate interfering substances for ELISA analysis (22,23). However, this might decrease the assay sensitivity and increase the limit of detection (LOD). Therefore, a basic requirement for sample dilution is high enough assay sensitivity, which allows detection at low analyte concentration. Biagini et al. (20,21) used a 1:10 dilution before the analysis of pesticide residues in urine by multiplexed fluorescence microbead covalent assay (FCMIA). For the ELISA analysis of metolachlor (24) and atrazine mercapturate (25) a dilution factor greater than 1:10 was needed to eliminate the interfering substances in the urine matrix. Urine samples analyzed for a chlorpyrifos metabolite, 3,5,6 trichloro-2-pyridinol (TCP) (26), and atrazine (27*)* required at least a 1:50 dilution. Elimination of the urine matrix effect required a substantial sample dilution (1:100 to > 1:1000) in the ELISAs for pyrethroid metabolites (18). Instead of dilution, Lyubimov et al. (28) took a simplified approach of using the ratio between 2,4-D-spiked and non-spiked samples to minimize the effect of interfering substances in urine.

For some assays, though, a more complete clean-up and elimination of interfering substances are required (13, 15, 29, 30). Since immunoassays tend to tolerate up to about 20 % of many organic solvents, the conventional sample preparation techniques like liquid-liquid extraction (LLE), and solid-phase extraction (SPE) can be easily coupled to immunoassays. SPE with C-18 reversed phase resin has been the method of choice in studies on urinary biomarkers (*31, 32*). In our studies on paraquat (17) and atrazine mercapturate (30) in human urine samples, SPE cleanup with a mixed mode strong cation exchange resin (Oasis-MCX) was required before ELISA. In some cases, immunoaffinity chromatography can be used for sample clean-up and concentration before immunoassay as demonstrated by Nichkova and Marco (33).

For blood analysis, only minimal sample preparation has been used prior to immunochemical analysis. For example, a competitive ELISA for the phenylurea herbicide chlortoluron (8) in human plasma required no sample pretreatment. Önnerfjord et al. (9) used a flow immunoassay with fluorescence detection for the measurement of triazine herbicides in human urine and plasma. For urine, a simple dilution was a sufficient pretreatment step but for the plasma samples, a SPE clean up with a restricted access (RA) C-18 column was needed in order to make the system more sensitive. In the study by Denovan et al. (10), saliva clean-up with solid phase extraction (SPE) using C-18 cartridges was necessary in order to minimize the saliva matrix effects detected previously (11).

Specific Examples from UC Davis

Assays for Insecticide Metabolites – Pyrethroid Metabolites

Pyrethroids are highly potent insecticides that have been widely used in agriculture, forestry, horticulture, animal and public health, and in households (34, 35). Out of all pyrethroids, the most common one, permethrin, is also used as the active ingredient in personal care products, such as shampoos and lotions for lice (36). Although pyrethroids are considered safe for humans because of their relatively low mammalian toxicity, numerous studies have shown that very high exposure to them might cause potential problems such as suppressive effects on the immune system, endocrine disruption (37), lymph node and splenic damage, and carcinogenesis (38). Kolaczinski and Curtis (39) have recently reviewed the debate on chronic illness as a result of low-level exposure to synthetic pyrethroid insecticides.

In mammals, pyrethroids are metabolized rapidly by oxidation and hydrolytic cleavage of the ester linkage, followed by various species-dependent conjugations such as to glucuronide, glycine, taurine, and sulfate (40,41) (Figure 1). Although no study has specifically determined the nature of the conjugates of pyrethroids in humans, it has been well established that glycine is the most

common amino acid used in conjugation reactions with xenobiotics containing a carboxylic acid group (42). Thus, the effort of some researchers in our laboratory has been focused on the development of immunoassays for the glycine conjugates of the respective pyrethroid metabolites, for example immunoassays for s-fenvalerate acid (sFA)-glycine as a biomarker for esfenvalerate exposure (32) and for the glycine conjugate of *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane (DCCA), the major metabolite of permethrin (43).

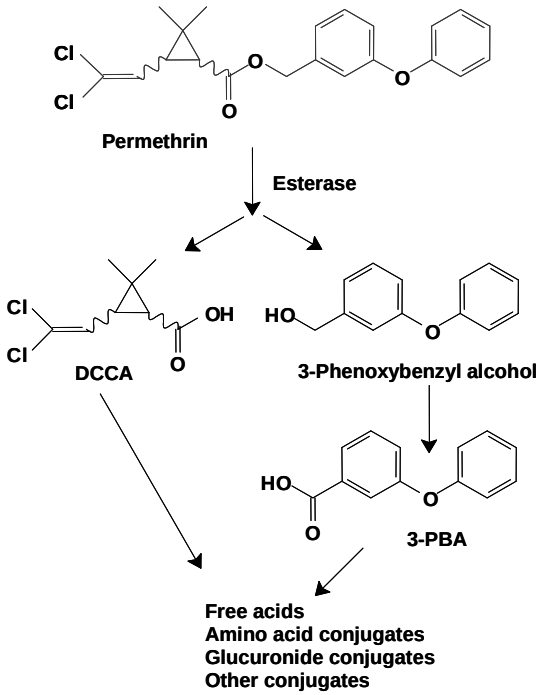

Figure 1. Metabolism of permethrin in mammals (40, 41)

The competitive indirect ELISAs for the detection of esfenvalerate metabolites (sFA-glycine and PBA-glycine) in human urine feature linear ranges for the optimized standard curves of approximately 0.03-60 ng mL^{-1} and 0.04-50 ng mL^{-1}, respectively. Both immunoassays are highly specific, and the lack of crossreactivity with the free PBA and FA makes these assays very useful for selective detection of esfenvalerate metabolites. The ELISAs were applied to the quantitative detection of trace amounts of sFA- and PBA-glycines in human

urine by using either direct dilution (150-fold) or a C18-SPE method. The introduction of the C18-SPE followed by 50-fold dilution allowed a limit of quantification (LOQ) of 1 ng mL^{-1} of sFA- and PBA-glycines. Both assays were validated in a blind fashion for 15 urine samples from individuals with no known exposure to pyrethorids, and an excellent correlation between spiked and measured concentration by the ELISAs was observed (32).

Another type of immunoassay, homogeneous fluoroimmunoassay for PBA-glycine, using the polyclonal antisera has also been reported (44). This quenching fluoroimmunoassay (QFIA) is based on the competition between a fluorescein-labeled and a non-labeled glycine conjugate of PBA. The assay offers an LOD of 0.25 ng mL^{-1} in buffer. Background fluorescence from urine samples was eliminated by 1000-fold sample dilution, which limits the applicability of this method to the detection of pyrethroid metabolites in pest control operators who may have been highly exposed. The average analytical recovery obtained for 12 spiked urine samples was 85-111%. Several factors, such as the lack of washing steps, short incubation of the immunoreagents (25 min) and the fast measurement (5 s) make the assay attractive for a rapid screening method to separate samples that can be further analyzed by more sensitive instrumental or ELISA methods.

The toxicity of the insecticide permethrin is dependent on its three-dimensional configuration. The *cis*-isomer is more toxic than the *trans*-isomer. However, *trans*-permethrin predominates (60-75%) in the commercial product, and the amount of free *trans*-DCCA in human urine ranges from 65 to 87% (45,46). With the aim to detect the *cis/trans*- DCCA metabolites several sensitive ELISAs with a heterologous configuration (*cis*/*trans* and *trans*/*cis*) between antibody specificity and hapten structure of the coating antigen have been reported recently (43). The IC50 values are as low as 1.3-2.2 ng mL^{-1} for *trans*- DCCA-glycine and 0.4-2.8 ng mL^{-1} for *cis*- DCCA-glycine in buffer. Among these assays the best combination for the detection of *cis/trans*- DCCA-glycine has been chosen for further optimization and application to urine samples (47). The quantitative and sensitive detection of DCCA-glycine by the ELISA in urine samples was achieved after C18-SPE clean up and further 5-fold dilution that completely removed the urine matrix interferences. This method has a LOD of 1 ng mL^{-1}. The method was validated by the ELISA analysis of urine samples from 12 non-exposed individuals spiked with a mixture of *cis/trans*- DCCA-glycine (40:60), and very good correlation between spiked and measured was observed (R^2=0.98).

Since most pyrethroids contain the phenoxybenzyl group, monitoring the common metabolite, 3-PBA, in urine would allow the evaluation of the human exposure to all pyrethroids containing this moiety. This was the objective of the development of a 3-PBA immunoassay by Shan et al. (18). This competitive ELISA obtained had a dynamic range of 0.1-5 ng mL^{-1} with an IC50 value of 1.65 ng mL^{-1} 3-PBA in buffer, which compares well with chromatographic

methods reviewed by Aprea et al. (2). The 3-PBA immunoassay is highly specific for the target analyte PBA and the related cyfluthrin metabolite (4-fluoro-3-phenoxybenzoic acid). The crossreactivity with parent pyrethroids and other metabolites is negligible. Urine matrix effects were eliminated by a simple 100-fold dilution prior to ELISA and the linear regression analysis of ELISA results of spiked urine samples from non-exposed people showed a good correlation (R^2=0.900). Furthermore, a good correlation between ELISA and GC-MS values was achieved in samples from exposed workers suggesting that the PBA immunoassay is useful for human exposure monitoring and toxicological studies.

Assays for Herbicides – Paraquat

Paraquat (1,1'dimethyl-4,4' bipyridinium) is a fast-acting, quaternary ammonium, non-selective, contact herbicide, which inhibits photosynthesis when applied to plant foliage. It is used extensively for both weed control and as a pre-harvest desiccant and defoliant. Although paraquat is highly water soluble, it is not easily leached from soil or taken up into plant root systems as it is quickly and strongly adsorbed to clay and soil organic matter. The extensive research on the fate of paraquat in agroecosystems has been reviewed by Summers (48) and recently by Roberts et al. (49).

Determination of the paraquat concentration in urine is a valuable tool for diagnosis in accidental, suicidal, and occupational intoxications, and it can be used for biological exposure assessment as well. In mid 1980's, Van Emon et al. (50) developed a competitive enzyme-linked immunosorbent assay (ELISA) for measurement of paraquat in human exposure samples. In comparison with a gas chromatographic method, the ELISA gave higher recoveries, was less labor intensive, and was more sensitive (LOD 0.1-1.0 ng mL^{-1}). This same method was recently used for paraquat analysis in an epidemiological study conducted by UCD in 2001-2003 (17).

This SALUD (Study of Agricultural Lung Disease) study tested the hypothesis that a low-level paraquat exposure can have adverse health effects including restrictive lung function. The study population included a total of 338 farm workers in Costa Rica, both pesticide handlers and non-handlers. In the same study, the paraquat ELISA was also used for measurement of paraquat trapped in air filters simulating the potential for exposure through inhalation. Prior to analysis, interfering substances in the urine samples were removed using Oasis-MCX (mixed mode cation exchange resin) SPE. When the results obtained by ELISA were validated against a current LC-MS method, the correlation between results for blind samples obtained using ELISA and LC-MS was significant (R^2 = 0.945 and 0.906 for spiked and field samples, respectively). This ELISA method had a limit of quantification of 2 ng mL^{-1} which is 5-fold lower than obtained with LC/MS/MS methods published

recently (51). The paraquat ELISA was able to distinguish farm workers who were exposed from those non-exposed (Table 1). For comparison, for the air filter analysis, paraquat was first extracted by 9 M H_2SO_4 at 60 ^oC for 12 hours, and the results obtained by ELISA showed good correlation (R^2 = 0.918) with the UV (256 nm) measurements (17).

Table I. Amount of paraquat excreted in urine (μg 24 h^{-1}) in samples analyzed by ELISA

Group	*Number of Samples*	*Paraquat (μg 24 h^{-1})*	*Range (μg 24 h^{-1})*	*% of Samples lower than LOQ*
Control 1	30	-	-	100.0
Control 2	53	0.31	0 – 6.8	92.5
Handler	119	5.64	0 – 75.4	47.0

NOTE: Control 1: Farm workers on control farms where no paraquat was used
Control 2: Farm workers not handling paraquat on farms where it was used
Handler: Farm workers who handle paraquat on farms where it was used
SOURCE: Data are from reference 17

Assays for Herbicide Metabolites - Atrazine Mercapturate

Atrazine is one of the most widely used herbicides in the United States. Due to its fairly good mobility in soil, it is one of the main surface water contaminants in the Midwestern United States (52,53). Atrazine has a low toxicity to humans but it has been implicated as a clastogen, an agent that causes chromosomal damage (54) and quite recently, atrazine at environmental concentrations has been found to have adverse effects on the development of anuran larvae (55). Despite its low acute toxicity to humans, atrazine is a potential endocrine disrupter and possible carcinogen, which poses a health risk to humans, especially to agricultural workers through occupational exposure.

Due to its rapid detoxification, atrazine metabolites are more likely to be found in urine and feces than the parent compound (56,57). The main urinary metabolite, atrazine mercapturate, (N-acetyl cysteine derivative of atrazine) (22) is quite stable and hence, can be used as a biomarker for atrazine exposure in humans (58). The relevance of urinary atrazine mercapturate (AM) in human metabolism has been confirmed using high-performance liquid chromatography–accelerator mass spectrometry (HPLC-AMS) to detect urinary atrazine metabolites after a dermal exposure to ^{14}C-labeled atrazine (58).

An enzyme-linked immunosorbent assay (ELISA) for AM was first developed in our laboratory by Lucas et al. (22). The assay was based on a

monoclonal antibody, and was able to detect AM down to 0.5 ng mL^{-1} in crude urine diluted to 25 % with buffer. Similarly, an ELISA based on a polyclonal anti-AM antibody offered a limit of quantification of 0.3 ng mL^{-1} after a simple (1:4) sample dilution (23). Because the levels of urinary metabolites measured in epidemiological studies are usually quite low, high assay sensitivity is often required. We attempted to improve the sensitivity of the atrazine mercapturate-ELISA using different SPE pretreatment methods, and compared them with sample dilution (30). For data validation, a new HPLC/MS method using on-line SPE and column switching was also developed for the analysis of atrazine mercapturate in human urine samples. Methods were further assessed and validated using a set of field urine samples collected in the National Cancer Institute (NCI) corn farming study.

Of the two SPE resins tested, the mixed-mode resin Oasis-MCX was more compatible with immunochemical analysis than the reversed-phase Oasis HLB. On the other hand, the HLB resin performed well as an HPLC precolumn. Obviously, atrazine mercapturate containing both hydrophobic and hydrophilic moieties is a challenging analyte for solid phase extraction. This finding indicates that for analytes like AM, a resin suitable for an LC/MS online SPE does not necessarily offer the best separation for ELISA, which requires more complete elimination of structurally related, interfering substances.

Validation of all three methods, LC-MS, ELISA+SPE, and ELISA+sample dilution with spiked urine samples showed good correlation between the known and measured concentrations with R^2 values of 0.996, 0.957 and 0.961, respectively (Figure 2). Overall, both ELISA methods tended to underestimate the urinary AM concentration (slopes = 0.84-0.86), which is probably an indication of an incomplete recovery of AM during the SPE clean-up. When a set (n=70) of urine samples from a corn farming study was analyzed, there was a good agreement (R^2 = 0.917) between the ln-transformed values obtained by ELISA+SPE and LC-MS suggesting that both methods would be suitable for the analysis of urinary AM as a biomarker for human exposure of atrazine. Both methods have similar limits of detection (SPE+ELISA 0.04 ng mL^{-1} with a 1-mL sample, LC/MS 0.05 ng mL^{-1} with a 10-µL sample), which are 5-10 fold lower than the ones previously reported in the literature (22,23,59).

Future directions

As already mentioned, the development of sensitive immunoassays for human biomonitoring has been quite intense during the past decade. However, most field studies using immunochemical analysis have been targeting industrial chemicals, not pesticides. One of the limiting factors for the analysis of pesticide residues in large-scale field studies is probably the lack of commercially available, affordable immunoreagents and kits for pesticide analysis.

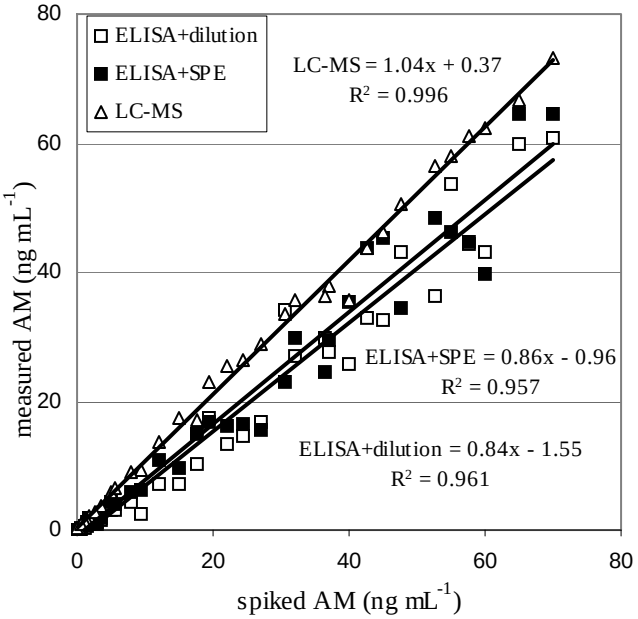

Figure I1. Correlation between known and measured concentrations of AM in urine samples obtained by ELISA or LC-MS. Data obtained from ref 30.

Another factor limiting the use of immunoassays in field studies is the low throughput of regular 96-well plate assays. In order to increase both throughput and sensitivity of immunochemical analysis, a concept of chemiluminescent immunoassay has been tested in our laboratory (60). The system is based on a chemiluminescent reporter, acridinium, which gives a detectable signal in less than 2 seconds. Acridinium label used in combination with an automated (ACS 180, Bayer) analyzer for the detection of 3-PBA in urine resulted in a decrease in analysis time and a substantial increase in sensitivity. The automated system was able to analyze 100 samples in one hour with a 5-fold increase in assay sensitivity (IC50 of 0.30 ng/mL). Another advantage of this system is the saving of immunoreagents because of the lower concentration of antibodies required for binding reactions.

One approach for increased throughput in pesticide immunoanalysis is the development of lateral flow (dip stick) assays (61,62) or immunosensors combined with micro-channels (63) or flow-injection immunoaffinity analysis (64). A recent article by Seydack (65), presents a good overview on the

development of biosensors based on nanoparticle labels and optical detection methods. Microfluidic lab-on-a-chip systems target biomarkers in physiological fluids with reduced sample, reagent, and assay time requirements, and therefore promise to have a significant impact on exposure analysis, especially in the field setting. The lab-on-chip systems are usually amenable to full automation and allow multiplexing of more than one target analyte but at the moment, they are still not used in practical applications.

A common trend in the human exposure analysis is a requirement for increased assay sensitivity to allow for detection of parent compounds or metabolites in the low parts per billion (ppb) range. One of the approaches for more sensitive assays has been the substitution of enzyme labels with more sensitive fluorescent probes (for a review see ref. 19). To overcome the inherent problem of high background fluorescence signals from biological material, probes that involve longer (far-red) wavelengths or longer fluorescence life-times seem to be most promising. Lanthanide chelates and lanthanide oxide nanoparticles have been successfully used as reporters in pesticide immunoanalysis (*14, 60,* 66 67). The advantages of europium oxide nanoparticles as fluorescent reporters include a large Stokes shift which decreases interference from scattered light, a sharp emission peak at the far-red (613 nm), a long-lifetime emission enabling time-gated detection and resistance to photobleaching.

Work by Ahn et al. (67) showed that the sensitivity of an immunoassay for the pyrethroid metabolite, 3-PBA, was increased by using europium oxide nanoparticle labels conjugated to the antigen. The assay was based on magnetic separation, and with an IC50 of 20 pg mL^{-1} showed about 1000-fold increase in sensitivity compared to the conventional 96-well plate assays with an enzyme label. Recently, Nichkova et al. (68) were able to demonstrate the use of biofunctionalized (IgG-PL-Eu:Gd_2O_3) nanoparticles as reporters in an indirect competitive fluorescence microimmunoassay for 3-PBA. Microarrays of BSA-PBA are fabricated by microcontact printing in line patterns (10 x 10μm) onto glass substrates. Confocal fluorescence imaging combined with internal standard (fluorescein) calibration was used for quantitative measurements. The non-optimized competitive microarray immunoassay had sensitivity in the low ppb range, which is similar to that of the conventional ELISA for 3-PBA. This work suggests the possibility for applying lanthanide oxide nanoparticles as fluorescent probes in microarray and biosensor technology, immunodiagnostics and high-throughput screening.

Other examples of promising high-throughput immunochemistry applications include chemiluminescent immunoassays based on microformat imaging using a charge-coupled device (CCD) camera (69). Ramanathan et al. (70) were able to use a portable module based on a photomultiplier tube (PMT) for the detection of pesticides in the field. More recently, Bhand et al. (71) used a novel immuno-array strategy for multicomponent analysis of two classes of

pesticides (triazines and phenoxyalkanoic acids). The approach was based on cross-reactive arrays of specific antibody pairs coupled to chemometric pattern recognition. Undoubtedly, systems and devices first developed for monitoring pesticides and their metabolites in the environment (19, 72) will be well suited for human biomonitoring as well, making immunoassays even more valuable tools for human exposure studies.

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