Bioanalytical Methods for Food Contaminant Analysis

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Foods are complex mixtures of lipids, carbohydrates, proteins, vitamins, organic compounds and other naturally occurring compounds. Sometimes added to this mixture are residues of pesticides, veterinary and human drugs, microbial toxins, preservatives, contaminants from food processing and packaging, and other residues. This milieu of compounds can pose difficulties in the analysis of food contaminants. There is an expanding need for rapid and cost effective residue methods for difficult food matrices to safeguard our food supply. Bioanalytical methods are established for many food contaminants such as the mycotoxins, and are the method of choice for many food allergens. Bioanalytical methods are often more cost-effective and more sensitive than instrumental procedures. Recent developments in bioanalytical methods may provide more applications for their use in food analysis.

A perhaps somewhat fictionalized, but vivid description of food production in the 1900s spurred then President Theodore Roosevelt to initiate the passage of the first federal law aimed at protecting the safety of the nation's food supply. While eating breakfast one morning the President read:

"There would be meat that had tumbled out on the floor, in the dirt and sawdust, where the workers had tramped and spit uncounted consumption germs. There would be meat stored in great piles in rooms; and the water from leaky roofs would drip over it, and thousands of rats would race about on it . . . These rats were nuisances, and the packers would put poisoned bread out for them; they would die, and the rats, bread, and meat would go into the hoppers together." (1). The original 1906 Food and Drugs Act and the Meat Inspection Act were shortly enacted, leading to the Federal Food, Drug, and Cosmetic Act of 1938, the 1947 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), which was replaced by the Federal Environmental

Pesticide Control Act, authorizing the U.S. EPA to review and register pesticides for specified uses, and more recently the Food Quality Protection Act (FQPA) (2, 3, 4).

Food Quality Protection Act

The National Academy of Sciences (NAS) major report "Pesticides in the Diets of Infants and Children" recommended a revision of U.S. pesticide laws to make foods safer for children (5). The report ultimately resulted in the Food Quality Protection Act (FQPA) of 1996 that was unanimously passed by both houses of Congress (4). The FQPA is based upon the report's recommendation to reduce exposure to pesticides in foods, particularly for vulnerable groups. The FQPA mandates much stronger standards for protecting public health from the hazards of pesticides in foods, and in part, puts special emphasis on ensuring that pesticide residues are safe for infants and children as their physiological and developmental stages may make them more susceptible to xenobiotics. The law requires the U.S. EPA to review all pesticide data, tighten exposure limits to make them safer for young children, and to look at all routes and sources (i.e., food, air, water, pets, homes, day care centers, schools, etc) when setting limits on the amount of pesticides that can remain on food. Dietary and non-dietary exposures must now be considered in an integrated manner. The law also establishes a single health-based standard for both processed and raw foods, while defining safety as a reasonable certainty of no harm to public health. The Academy report recommended that pesticide residue monitoring programs target foods particularly consumed by children, and that the analytical testing methods used be standardized, validated and subject to strict quality control and quality assurance programs (5).

This aggregate exposure approach clearly requires cost-effective analytical methods for determining pesticide residues in a variety of matrices.

Bioanalytical Methods Development and Applications

The full impact of bioanalytical methods on food analysis remains to be seen as methods become more refined, new techniques developed, and reagents become readily available. The U.S. food supply is one of the safest in the world, but to maintain this safety a vast array of analysis must be performed on a routine basis. In a global society where raw materials and foods for human consumption are easily exchanged among many countries before a final product is obtained, analytical methods are particularly critical to protecting human health. Analytical methods must be cost-effective, adaptable, dependable, and produce data of known quality. Analytical chemists are ever challenged to keep pace with the latest food contaminant issue be it a pesticide, food additive (i.e., colorant, flavoring, preservative), antibiotic, hormone, a misplaced pharmaceutical or environmental contaminant (i.e. dioxin, PCBs, melamine, phthalates), natural toxin, or microorganism. This is no small issue especially given that food matrices can be extremely complex and the definitive answer is typically required immediately and for pennies on the dollar.

Bioanalytical methods such as immunoassays, immunosensors, and immunoaffinity chromatography are providing information regarding the presence and concentration of contaminants that may impact human health and the environment (6). Immunochemical methods can be used for both sample preparations (i.e., extraction, cleanup and concentration) and

detection. Methods specific for food analysis have been reported, while methods developed for other matrices could be adapted for foods (7, 8, 9, 19, 11, 12).

Immunochemical detection was first applied to clinical situations for diagnostic purposes. The sensitivity and selectivity of the interaction between antibodies and their typical large target antigens (i.e., hormones, bacteria, toxins, etc) was aptly used in highly successful diagnostic methods for medical and health-care applications. To stimulate the immune system for generation of specific antibodies the target must be ≥10,000 daltons. The development of specific antibodies for small molecules (<200 daltons) can be obtained through the use of a carrier molecule. Pesticide residue chemists astutely recognized the potential of the technology for small molecule detection in the 1970s and advances in the technology have been reported ever since for a variety of environmental contaminants (6, 13, 14, 15). Regardless of the molecular weight of the analyte, immunochemical methods are based on selective antibodies combining with a particular target or members of a closely related analyte group.

The detailed development and applications of many bioanalytical techniques, as well as, quality assurance measures, and how to integrate bioanalytical methods into an instrumental analytical laboratory have been extensively reviewed (6). Described here are current applications of immunochemical methods for foods; methods that could be applied; new developments in reagents, formats and labels; and the use of nanotechnology, as well as, the potential problem these small particles may bring.

Reagents for Bioanalytical Methods

The interactions between an antibody and its target, and cross-reacting compounds, are extremely complex. Rational hapten design originally entailed studying the structure of the target analyte and synthesizing a hapten that closely resembled the target. A newer approach for hapten design is based on computer-assisted molecular modeling (CAMM) (16). Quantitative structure-activity relationship (QSAR) models provide insight into the mechanism of antibody binding by illustrating structural as well as electronic features important to the binding event. QSAR data for target analytes can aid in the design of haptens that mimic the analyte in key areas in an effort to quantitatively produce antibodies of high affinity and specificity (17). Molecular and quantum mechanics modeling programs give 3-dimensional conformations and electrostatic potentials that can estimate the influence of spacer arms and hapten attachment. Many programs are easy to use yet provide in-depth information on bond lengths, bond angles, ionization potentials, electron affinities, minimum energy conformations, and other molecular data. Antibody binding sites, and antibody-antigen interactions can even be modeled, and cross reactivity data analyzed to design additional haptens or optimize assay conditions (17). Haptens for several compounds of interest to food safety (e.g., parathion, permethrin, simicarbazide) have been modeled for hapten design, and to explain cross reactivity (18, 19, 20). These computation chemistry programs can assist in developing the desired antibodies, but the immune system can still surprise analytical immunochemists.

Ever since Köhler and Milstein (21) described the first monoclonal antibody production, there have been improvements and permutations on the concept. Antibody engineering has become a well-developed discipline providing a vast array of tailor-made antibody reagents (22, 23). Recombinant antibody fragments, serum-free production, and chimeric antibodies are other advances in reagent development (23, 24). Detailed studies of antibody structures, using time-

of-flight mass spectrometry (TOF MS), have provided insight into antibody binding for hapten design (25).

Antibody mimics, such as molecular imprinted polymers (MIPs), have proven useful in many bioanalytical methods. MIPs are developed by forming, a complex between a functional monomer and the template (analyte) molecule. A cross-linking monomer is used to impart a degree of rigidity to the created receptor. Molecular imprinting for small molecules has been described in detail (26). Molecular imprinting of sol-gel thin films provides advantages over organic-polymers, yielding reagents with better kinetics, low nonspecific adsorption and high association constants (27). Computational software can be used to model the MIP with its target to determine the binding performance prior to its use. These artificial antibodies have been developed for the selective recognition of pesticides, veterinary drug residues and other contaminants that may be found in food (26, 28, 29, 30).

Nucleic acids with defined sequences and structures can also be used as affinity probes (31). Aptamers and molecular beacons are two types of nucleic acid probes that are more stable than antibodies. Aptamers are single stranded DNA or RNA ligands that can be selected for different targets. These nucleic acids are able to fold into a well-defined 3-D structure, showing high affinity and specificity for target molecules. Once an atpamer is selected, cloned and sequenced, it can be routinely synthesized and easily amplified using polymerase chain reaction technology, providing ample reagent for bioanalytical methods. Aptamers have been primarily applied to large molecules but have also been developed for vitamins, antibiotics and ochratoxin A, among other small molecules (31).

The development of phage peptides capable of recognizing the conformational change of an antibody-binding pocket upon binding to analyte, allowed the development of noncompetitive

immuoassays for small molecules (32). In a model system, a polyclonal antibody for 3-phenoxybenzoic acid (3-PBA) and an anti-immunocomplex phage clone were used in a magnetic bead-based assay to detect the urinary biomarker at low ng/mL levels (32). This method could be extended to food matrices providing additional sensitivity, speed and specificity over competitive immunoassays.

Immunoaffinity Chromatography

Immunoaffinity chromatography can provide sample enrichment and cleanup prior to instrumental or immunoassay detection (33). The technique is part of the green chemistry movement, utilizing low levels of organic solvents and accommodating small sample volumes. The resulting extracts can be coupled with immunochemical or instrumental detection.

The entrapment of antibodies in a ceramic SiO_2 sol-gel matrix is finding applications for clean-up and concentration of target analytes from agricultural samples (34). A sol-gel based immunoaffinity purification (IAP) column for the pyrethroid bioallethrin was coupled to immunoassay detection for food samples (35). Antibodies entrapped in the sol-gel matrix were able to bind bioallethrin as the sample extract was loaded on the column. An elution step releases the analyte from the antibodies. The antibodies remain on the column for another sample run. The sol-gel IAP followed by solid-phase sample concentration was effective in removing interfering components and resulted in high recoveries of bioallethrin from spiked crude acetonic extracts of fruits and vegetables. The IAP column could tolerate a high concentration of sample extract (28%) without breakthrough. Solid-phase treatment alone failed

to remove the interfering components from the samples as determined by GC/MS. A schematic representation of the IAP process is shown in Figure 1.

Immunoaffinity column sample cleanup, for mycotoxin food analysis, has become a routine practice especially since there is a choice of commercial columns (36). The technique has also been applied to veterinary drugs, phycotoxins, vitamins, process contaminants and pesticides (37). An atrazine immunoaffinity column provided efficient cleanup for composite food samples prior to ELISA or GC/MS analysis (38). The procedure was highly quantitative and more streamlined than the GC/MS procedure. As immunoaffinity chromatography is rather reagent intensive, aptamers and MIPS are being investigated for use in affinity cartridges. A MIP affinity solid phase extraction cartridge for parathion for the direct extraction of parathion from water samples could have applicability for food samples (39).

Within the U.S. EPA, the National Exposure Research Laboratory conducts studies to characterize exposures via various routes in keeping with the requirements of the FQPA. Dietary sampling usually requires analysis of a composite food sample, composed from duplicates of all solid food items consumed during a 24-hour monitoring period. These samples may be a combination of grains, fruits, vegetables, meats and/or dairy products. Composite food samples usually require a rigorous extraction, followed by several clean-up steps employing large amounts of organic solvents. To streamline sample preparations and minimize organic solvent usage, permethrin-specific MIP solid-phase extraction cartridges were used for the cleanup of pressurized liquid extraction (PLE) extracts of composite food samples (40). The MIP immunoaffinity process greatly reduced sample preparation time while partially eliminating the PLE co-extractives. The MIP procedure enabled detection of the permethrin isomers in the low

ug/kg range as detected by gas chromatography/mass spectrometry (GC/MS). An immunoassay is being developed to analyze the PLE-MIP extracts

Immunoassays

Many immunoassay methods have been reported for detecting pesticide residues and other contaminants in food matrices (Tables 1 and 2), while many antibodies for other pesticides have been reported but have not yet been applied to food analysis (12). Many immunoassay formats have been developed, with one of the most common, being the enzyme-linked immunosorbent assay (ELISA). The steps in the development of an immunoassay, procedures for preparing buffers and quality assurance considerations can be found in (6). Examples of indirect and direct competitive ELISA formats are shown in Figure 2. Other formats using multiplexed fluorescence microbeads provide multianalyte capability with the use of flow cytometry (60).

ELISAs can be used to monitor the safety of food from raw material production to final product or even follow pesticide concentrations through trophic food chains (61). An ELISA monitored atrazine levels in soil (3.2 – 85.4 ug/Kg), vegetable roots (32.9 – 148.9 ug/Kg), green parts of plants (67.7 – 136.4 ug/Kg), cereals (42.4 -91.5 ug/Kg) and meat (1.3 – 8.4 ug/Kg) (61). A correlation of 0.97 was obtained between the ELISA results and a HPLC procedure. The study also illustrated the easy utilization of immunoassay methods by a food laboratory that did not typically perform these types of analysies.

Immunoassays are becoming the method of choice for analytes difficult to analyze by instrumental methods. ELISA is considered the gold standard for measuring clenbuterol (56). Clenbuterol, a β-adrenergic agonist growth promoter, has been associated with severe food

poisoning outbreaks and is included in several food monitoring programs. A microsphere-based competitive fluorescence immunoassay with a sensitivity of 0.01 ng/mL, provides a much more streamlined analysis than a mass spectrometry procedure, enabling more samples to be processed in a shorter timeframe. The food borne pathogen *Listeria monocytogenes* and the toxin listeriolysin O is routinely analyzed by immunochemical methods in various food commodities (62). The multi-detection of mycotoxins by fluorescence polarization immunoassay in food samples is becoming a standard procedure (63). Quinolones have been used in human and veterinary medicine for over a decade, and may be found in edible animal products (57). The low detection levels required and the complexity of food matrices demand highly sensitive and selective methods, including immunoassays and biosensors which have been the subject of a review (57).

Several examples appear in the literature where immunochemical methods are either being developed to replace long and tedious procedures, or are used as screening methods in a tiered approach to more effectively use analytical instrumentation. Nitrofuran drugs have potentially harmful effects on human health and must be monitored for illegal residues. Semicarbazide, a metabolite of nitrofurazone (a broad-spectrum anti-bactericide drug), is used as a marker residue for illegal use of this drug in food producing animals. To detect SEM residues, food samples undergo analysis by LC-MS that requires a lengthy sample preparation. To reduce costs and increase sample throughput, sensitive antibodies (0.2 ng/mL) were developed for screening food samples for SEM in an ELISA format (64). The GC method for detecting the insecticide triazophos in agricultural products is time consuming (65). In an effort to streamline triazophos analysis, specific antibodies were produced for immunoassay development to test agricultural commodities.

Rapid field-portable methods enable on-site monitoring of pesticide products. A simple lateral-flow immuno-chromatographic dipstick verified the label claim of active ingredients in commercial pesticide formulations of endosulfan and the type-II pyrethroids cypermethrin, deltamethrin, and fenvalerate on a single strip (66). Two intensely colored purple lines appear when the strip is dipped in a solution of substandard pesticide formulations (66). Concentrations of endosulfan (1800 ug/L), cypermethrin (800 ug/L), deltamethrin (1000 ug/L), and fenvalerate (1400 ug/L) are able to inhibit the appearance of either of the two lines when dipped into a solution of the correct pesticide formulations. A similar method employing gold-based lateral-flow strips has been reported for the simultaneous detection of carbofuran and triazophos with detection limits of 32 and 4 ug/L (11).

Complex matrices, such as food, require extensive sample extraction and purification. Immunoassays have been coupled with many extraction techniques (i.e., Soxhlet, pressurized liquid extraction, solid phase, sonic, super critical, etc) although a solvent exchange step may be required. Pressurized liquid extraction methods, reported for extracting pesticides from many food commodities, typically yield colored extracts which are not an issue for immunoassay detection (67, 68, 69).

An immunoassay for the detection of 3,5,6-TCP in composite food samples from a duplicate diet study, provided a more streamlined approach in comparison to a GC/MS procedure (33). The ELISA employed a simple extraction using a vortex mixer, while the standard GC/MS procedure employed PLE. The PLE extracts gave unsatisfactory ELISA results, possibly because of the residual fatty acids and fatty acid esters present. Although different sample preparation procedures were used for the ELISA and GC/MS analysis the data were generally in good agreement over a concentration range of 2.28 to 11.2 ppb (33). Other duplicate diet

samples were extracted using PLE followed by a solid-phase cleanup, and analyzed for chlorpyrifos using an ELISA. Good agreement was obtained between the GC/MS and the ELISA data (70).

Several immunoassay methods have been developed for a class-specific semi-quantitative approach. Many organophosphorous pesticides (OPs) have a common phosphate or thiophosphate group along with a specific ring or alkyl chain. As the OPs are an important class of pesticides many efforts have been made to enable a class selective analysis, such as the use of a generic hapten to produce broadly selective antibodies (71). A generic hapten, 4-(diethoxyphosphoro thioyloxy) benzoic acid produced a polyclonal antibody with a high sensitivity to seven common O,O-diethyl organophosphorous pesticides. In a competitive indirect ELISA, IC_{50} values ranged from 13 ng/mL to 1301 ng/mL for parathion, coumaphos, quinalphos, triazophos, phorate, dichlofenthion, and phoxim (17). Another approach for class-specific analysis is the use of heterologous coating antigens. A recent example is the use of the generic hapten 3-(4-dimethoxyphosphorothioyloxy phenyl) propanoic acid to produce monoclonal antibodies. Heterologous haptens were designed for use as coating antigens and gave lower IC_{50} values for six OPs (parathion, chlorpyrifos-methyl, fenthion, malathion, fenitrothion, and tolclofos-methyl) when compared to the homologous hapten coating antigen (72). Specific antibodies can also be mixed in an ELISA for a semi-quantitative screening method (73).

Immunoassays are typically evaluated against a standard chromatographic procedure for accuracy. Method validation protocols and statistical designs have been reviewed and evaluated (74). Appropriate statistical methods can estimate the false positive rate from a sufficient

number of replicates of negative control samples. Similarly, false negative rates can be estimated using low level positive control samples (75).

Microarrays

Until the advancement of microarrays, immunoassay analyses were typically for a single analyte or closely related analyte group. The common practice of applying pesticides as mixtures, results in simultaneous exposures to multiple pesticides requiring multi-analyte capability. Multi-analyte detection systems for simultaneous screening of pesticides, their metabolites, and degradation products can be configured on a single microarray.

The surface chemistry applied to the glass slide is an important aspect of the ELISA microarray platform and has been reviewed for many different commercially available slide types (76). A good surface chemistry for antibody immobilization must exhibit: (1) high binding capacity, (2) an ability to retain antibody activity, (3) low variability between slides, and (4) a high signal-to-noise ratio. Cross-reactivity between assays and nonspecific protein binding should be minimal. New fluorescent probes for microarray-based bioanalysis such as europium chelates, dye-doped silica nanoparticles, and quantum dots have all been applied to assays for the detection of agrochemicals.

Antibody microarrays are an emerging technology for the detection of small agrochemicals. Atrazine, nonylphenol, 17-beta estradiol, paraverine and chloramphenicol were simultaneously and quantitatively detected on an immunochip with a working range of 0.001 – 5 ug/mL (77). The five different specific antibodies could independently bind to their target for individual

analyte quantitation. The work illustrates the potential for on-line detection of multiple small molecules for food monitoring.

Atrazine and dichlobenil are broad-spectrum herbicides used in agriculture and urban settings. A microarray simultaneously detected atrazine and the dichlobenil degradate, 2,6-dichlorobenzamide (BAM) in reduced sample volumes with enhanced sensitivity (78). Using a fluorescently tagged monoclonal antibody, the level of detection for BAM was 1 ng/L and 3 ng/L for atrazine. The microarray was 20-fold more sensitive than a 96-well assay using the same reagents. Compared to a gas chromatography/mass spectrometry (GC/MS) analyses the microarray was 10 times more sensitive without sample concentration. Measuring both pesticides simultaneously did not affect assay sensitivity compared to the single analyte quantification, indicating the two antibodies did not interfere with each other in solution (78). More complex microarrays are built by adding additional antibody-antigen pairs and testing the performance of the microarray after each addition until a true multiplexed system is obtained.

Sensors

Biosensors may help to fulfill the need for fast screening techniques that can detect the presence of pesticide residues in food and beverage products prior to delivery to the consumer (79). Biosensors are analytic instruments composed of a recognition element (e.g., an antibody) and a transducer that converts the binding of the antibody with antigen into a measurable physical signal. A biosensor is able to detect analyte continuously and selectively, yielding a response in real time. Several types of immunosensors have been employed for pesticide detection including optical, evanescent wave, surface-plasmon resonance, fluorescence and

chemilumenscence. Screen-printed electrodes have been reviewed for pesticides, hormones and other potential food contaminants (9). Sensors have been used to detect novel genes in crop plants (80). Table 3 lists sensors that have been recently developed for food matrices. Sensors that have been developed for environmental monitoring could be applied to food matrices (91). Much research has been directed towards the development of sensors for food analysis and has been extensively reviewed (80). The application of biosensors for the detection of analytes, foodborne microorganisms, and bacterial toxins was recently reviewed, and concluded that the next generation of biotechnology-based industries could be nanoscale sensors and ultraminiaturized sensors to monitor a range of chemicals in foods (92).

Biomarkers of Exposure

Bioanalytical methods are being developed and applied for biomarker detection to aid in exposure assessment studies. Immunoassay methods were incorporated into a biological monitoring exposure study to detect biomarkers in the urine of exposed herbicide applicators providing data before and during the spraying season (93). Zirconium oxide (ZrO_2) nanoparticles were used as selective sorbents for an adsorption-based immunoassay coupled with an electrochemical quartz crystal microbalance for detecting phosphorylated acetylcholinesterase as a biomarker for OP pesticides (94). The technique is simple and sensitive, providing data in real time, for the onsite biomonitoring of exposures to OP pesticides (94). Microarrays to detect biomarkers in blood and urine for liver, cardiac damage or organ pathology are frequently employed during the pesticide development process (95).

Food allergens

Food allergens are typically proteins, and are a continuing challenge to food safety. Traces of a food allergen can cause an allergic reaction manifesting in a cutaneous, respiratory, gastrointestinal or systemic response, including fatal anaphylactic shock. There is clearly a need for rapid, robust, and cost-effective analytical methods of high specificity and sensitivity, to detect even traces of allergens.

Sensors can impact food safety programs by offering immediate, on-line detection and quantification of inadvertent allergens such as peanuts in food (96). ELISAs are currently the method of choice to determine allergens in various food commodities, encompassing raw materials, as well as intermediate and finished food products (96). Sensors and microarrays have found application to not only food allergen monitoring but also clinical studies of food-induced allergic reactions. IgE eiptope mapping of food allergens is now possible using microarrays. The characterization of food allergens will help to uncover the sequential and structural properties that determine the behavior of proteins as food allergens (24, 97).

World Health Organization Initiative

Foodborne diseases and illnesses encompass a wide spectrum and are a growing public health problem worldwide. The World Health Organization has an initiative to provide reliable and accurate estimates of the global burden caused by ingesting foodstuffs contaminated with chemicals, microorganisms and other contaminants (98). Part of the initiative is to provide countries with simple, user-friendly tools to conduct their own studies. Simple, but effective

immunochemical methods could provide data to determine the effectiveness of food safety protocols in non-laboratory settings.

Impact of Nanotechnology

Nanoparticles hold promising potential as bioconjugate probes for bioanalytical methods (99). Some researchers believe that nanotechnology is at the center of advancement for bioanalytical methods via their use as novel materials for labels and supports. Free-standing carbon nanotube arrays provided an efficient 3-dimensional platform for deposition of molecular imprinted polymers as demonstrated for an amperometric sensor to detect caffeine (100). Carbon nanotubes provided a 100-fold enhancement in the resulting electrochemical signal when compared to a system with a single-enzyme label (101).

Semiconductor quantum dots (QDs) are nanoparticles that have generated widespread research interest in analytical chemistry due to their unique optical and electronic properties. QDs have diameters between 2–50 nm with typical compositions of CdSe, CdTe, InP, and InAs. QDs are resistant to photobleaching and their size-dependent fluorescence wavelength, make them attractive labels particularly for multiplexed analysis. QDs have high emission yields, narrow spectral bands and have been described as having a tunable emission profile, as they can be tuned based on their size (Figure 3). A single wavelength can be used to simultaneously excite QDs with different sizes, and as a result different emission spectra are obtained (101) The surface of QDs can be conjugated to various affinity ligands such as antibodies to detect specific target analytes in immunoassays, sensors, arrays, flow cytometry, and other formats (102).

QDs were tested as labels in an immunoassay microarray for the multiplex detection of the pyrethroid biomarker, 3-phenoxybenzoic acid (3-PBA), and the atrazine biomarker, atrazine-mercapturate, (AM) (103). Microarrays were fabricated by microcontact printing of the two coating antigens onto glass slides. A solution of the specific antibodies containing the analytes, were incubated with the prepared slides. The two types of QDs used (QD580 and QD 620) gave separate fluorescence emission peaks upon UV excitation enabling the detection of the two analytes.

QDs have also been applied to the measurement of 2,4,D. The herbicide was measured based on the competitive binding between a 2,4-D-alkaline phosphatase-CdTe QD conjugate and free 2,4-D, with immobilized anti-2,4-D antibodies on an immunoreactor column. It was possible to detect 2,4-D at 250 pg/mL using fluorescence detection which provided better sensitivity than conventional methods (103).

The potential health effects of nanoparticles are still not clear and difficult to address properly. However, a few studies have revealed the cytotoxicity of QDs on different cell lines by using CdSe-core QDs as models (104). Given the increasing use of QDs, there is potential for environmental and human exposure to these and other nanomaterials. However, issues regarding the environmental fate and transport of QDs and other nanomaterials remain to be answered. There is a high possibility that human beings will be exposed to nanoparticles through inhalation, dermal adsorption and digestion. Pumpkin plants have been shown to uptake, translocate and accumulate manufactured iron oxide nanoparticles, indicating that plant uptake is a potential transport pathway of nanoparticles in the environment and may be a route for human exposure (105). Although nanoparticles hold promise for bioconjugate probes in many bioanalytical methods there are still challenges and issues in working with them as recently reviewed (106).

Conclusions

As described in the NAS report more data are needed to accurately determine the impact of pesticides on human health, especially for infants and young children. Uncertainties in the assessment of human exposures to pesticides and other contaminants can be reduced through indepth dietary exposure studies. An aggregate exposure approach, as required by the FQPA, must consider all sources and routes of exposure.

The impact of bioanlaytical methods on food analysis will continue, if advances proceed in several key areas. Antibodies or other recognition reagents must be commercially available for methods development. Provided the specific antibody is available and suitable extraction methods can be identified, any of the formats presented here could be used for detecting contaminants in food. The use of novel labels such as nanoparticles; the development of non-competitive immunoassays for more sensitive, simpler and rapid assays; and the development of formats for multi-analyte detection such as microarrays and microspheres must be further investigated. Relevant metabolites and degradation products for antibody development and hapten design must be identified (107).

Food products are complex mixtures making food analysis a complex issue. The quantitative analysis of food contaminants is essential with respect to safeguarding public health, preventing illicit use of compounds, and facilitating government regulations and surveillance (42). Bioanalytical methods have a definite role in this important endeavor.

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FIGURE CAPTIONS

Figure 1. General scheme of the sol-gel based immunoaffinity chromatography process. (Adapted from Alstein, M. & Bronshtein, A. (2007) in *Immunoassays and Other Bioanalytical Techniques*, J.M. Van Emon (Ed), CRC Books, Taylor and Francis Books, New York, NY, pp 357-383. With permission.)

Figure 2. Examples of indirect and direct ELISA formats (6).

Figure 3. Schematic diagram of quantum dots functionalized with pesticide haptens and their size dependent wavelength.

Table 1. Pesticide Immunoassays for Food Matrices

Analyte	Matrix	LOD	Assay Format	Reference
Carbofuran	Cabbage, carrot, green soybean	3.44 ng/ml	ELISA	42
Chlorpyrifos-methyl	Grape, Chinese cabbage	0.32 ng/ml	ELISA	43
Chlorpyrifos, diazinon, fenthion, and malathion	Olive oil	10-46 ng/ml	ELISA	44
DDT	Grape, mango juice, milk, cauliflower	27 ng/ml	Gold nanoparticle dipstick immunoassay	45
Fenitrothion	Grape, peach, pear, tomato	0.01 ng/ml	ELISA	46
Imidacloprid	Fruit juices	5-20 μg/L	ELISA	47
Iprodione	Apple, cucumber, and eggplant	0.3 ng/g	ELISA	48
Isofenphos	Rice, lettuce	4.8 ng/ml	ELISA	49
Metolcarb	Rice	0.08-0.10 ng/ml	ELISA	50
Parathion	Cucumber, rice	0.70 ng/ml	ELISA	51
Parathion	Potato, celery, Chinese cabbage	26 ng/ml	ELISA	52
Parathion	Tomato, cucumber, banana, apple, orange, pear and sugarcane	0.08 ng/ml	ELISA	53
Simazine	Orange juice, milk	0.1 ng/L	Liquid membrane assay cartridge	54

Table 2. Immunoassays for Food Contaminants

Analyte	Matrix	LOD	Assay Format	Reference
Acrylamide	Water	65.7 μg/kg	ELISA	55
Aflatoxins	Milk, peanut, animal feed	0.3-25 μg/kg	Lateral flow strip	36
Brevatoxins	Mollusks	0.6 ng/well	ELISA	8
Clenbuterol	***	0.01 ng/ml	Fluorescence microsphere	56
Quinolone	Milk, meat, fish	Low μg/kg	Various	57
Sudan 1	Chili powder/sauce, tomato sauce, and sausage	0.07-0.14 ng/ml	ELISA	7
Toosendanin	Chinese cabbage, tomato, apple	0.014 μg/ml	ELISA	58
Trichothecine mycotoxins	Milk, cereal	0.5-1 μg/kg	Immunoaffinity, ELISA	59

Table 3. Sensors for Food Contaminants

Analyte	Sensor	Matrix	LOD	Reference
Atrazine	Infrared optical	Buffer	4 ppb	81
Atrazine	Biocomposite transducer	Orange juice	0.006 μg/L	82
Atrazine	Impedimetric immunosensor	Wine grapes	8.34 μg/L	83
Atrazine	Conductimetric immunosensor	Wine	0.1-1 μg/L	84
Atrazine	Optical immunosensor	Wine	6.8 μg/L	79
Botrytis cinerea	Sreen-printed microfluidic modified with carbon nanotubes	Apple tissues	0.02 μg/ml	85
Carbaryl, 3,5,6-trichloro-2-pyridinol (TCP)	Piezoelectric	Orange and apple juice	11 μg/L (Carbaryl) 7 μg/L (TCP)	86
Chlorpyrifos	Plasmon resonance	Water	45-64 μg/L	87
Flumequine	Optical	Broiler serum, muscle	500 μg/g	88
Fluoroquinolones	Surface Plasmon Resonance (SPR)	Chicken muscle	0.5 μg/g	89
Quinolone, tetracycline	Electrochemical	Milk	25 μg/L	90