REVIEW

Biosensors for the analysis of microbiological and chemical contaminants in food

T. F. McGrath · C. T. Elliott · T. L. Fodey

Abstract Increases in food production and the ever-present threat of food contamination from microbiological and chemical sources have led the food industry and regulators to pursue rapid, inexpensive methods of analysis to safeguard the health and safety of the consumer. Although sophisticated techniques such as chromatography and spectrometry provide more accurate and conclusive results, screening tests allow a much higher throughput of samples at a lower cost and with less operator training, so larger numbers of samples can be analysed. Biosensors combine a biological recognition element (enzyme, antibody, receptor) with a transducer to produce a measurable signal proportional to the extent of interaction between the recognition element and the analyte. The different uses of the biosensing instrumentation available today are extremely varied, with food analysis as an emerging and growing application. The advantages offered by biosensors over other screening methods such as radioimmunoassay, enzyme-linked immunosorbent assay, fluorescence immunoassay and luminescence immunoassay, with respect to food analysis, include automation, improved reproducibility, speed of analysis and real-time analysis. This article will provide a brief footing in history before reviewing the latest developments in biosensor applications for analysis of food contaminants (January 2007 to December 2010), focusing on the detection of pathogens, toxins, pesticides and veterinary drug residues by biosensors, with emphasis on articles showing data in food matrices. The main areas of development common to these groups of contaminants include multiplexing, the ability to simultaneously analyse a sample for more than one contaminant and portability. Biosensors currently have an important role in food safety; further advances in the technology, reagents and sample handling will surely reinforce this position.

Keywords Biosensor · Food analysis · Pathogen · Toxin · Pesticide · Veterinary drug residues

T. F. McGrath (✉) · C. T. Elliott · T. L. Fodey
ASSET Technology Centre, Institute of Agri-Food and Land Use,
School of Biological Sciences, Queen's University Belfast,
David Keir Building, Stranmillis Road,
Belfast BT9 5AG, UK
e-mail: t.mcgrath@qub.ac.uk

Introduction

It is hard to believe that biosensors are almost 50 years old. After the International Union of Pure and Applied Chemistry defined what a biosensor was [1], and then further clarified its identity [2], it became widely acknowledged that Leland C. Clark Jr. was the "father of biosensors" [3]. Although in the 1960s they were referred to as enzyme electrodes [3–5], it was Karl Cammann who was attributed with creating the term "biosensor" [2, 3, 6].

According to Thevenot et al. [2], a biosensor is, in essence, a self-contained integrated device which consists of a biological component that is maintained in spatial contact with an electrochemical transducer. Since this definition, others have dropped the word "electrochemical", thus allowing the International Union of Pure and Applied Chemistry definition to represent other types of transducers [7–9].

In general, a biosensor works because the biological component interacts specifically with its target, causing some sort of signal change that is proportional to the target concentration. This signal change can be measured by the transducer [10].

The most common modes of classifying biosensors are according to their biological component or their transducer

type. Table 1 [2, 7, 8, 11–13] lists the most common classifications. The following references are recent articles that provide details on the principles of the different types of transducer: electrochemical [13–15], optical [15–18], mass/acoustic [15, 19, 20] and thermal [21].

Biosensors have been employed in a wide variety of areas. Table 2 provides a list of disciplines [10, 11, 22–24] that have utilised biosensors, with corresponding examples of their applications.

Biosensors in food analysis

A search of the ISI Web of Science database for the topic "biosensor" with publication dates between 2005 and 2010, and disregarding review articles, returns 12,155 hits [25]. Refining this search with the topic "food" returns 496 hits [26]. This indicates that only approximately 4% of the biosensor literature between 2005 and 2010 was food-related. Figure 1 shows that the number of food-related biosensor publications between the years was maintained at this relatively low level. The actual numbers of biosensor publications related to food contaminants will be lower since, as indicated in Table 2, biosensors are utilised within several food-analysis-related areas. This corroborates the findings of Cock et al. [10], who also mentioned the slow uptake of biosensors in the agri-food sector. The low number of publications and the low uptake of technology in this sector could be down to the following:

– The sector is very conservative and is results-orientated, not technology-orientated. The methods currently used

Table 1 Classification of biosensors

Biological component	Transducer
Enzymes	Electrochemical
Whole cells, organelles or tissue	Potentiometric
Antibodies	Amperometric
Nucleic acids	Conductometric
Receptors	Optical
Aptamers	Absorbance
Peptides	Reflectance
Lipids and micelles	Luminescence
	Refractive index
	SPR
	Mass/acoustic:
	Piezoelectric
	Cantilever
	Thermal

SPR surface plasmon resonance

Table 2 Examples of disciplines that have utilised biosensors

Discipline	Example
Medical diagnostics	Cancer detection [148]
Veterinary diagnostics	Detection of bacterial infection in hens [149]
Food safety	Detection of antibiotics in milk [150]
Food processing	Detection of bacteria in chicken carcass [58]
Food quality	Determination of vitamin concentration [151]
Agriculture	Screening for GMOs [152]
Bioprocessing	Maintenance and control of haematopoietic stem cell cultures [153]
Environmental monitoring	Measuring changes in plankton populations to monitor climate change [154]
Industrial monitoring	Wastewater [155]
Antiterrorism	Detection of explosive substances [156]
Life sciences/drug discovery	Screening for therapeutic antibodies [157]

have worked sufficiently well for years and so there is no perceived benefit in change.
– The sector often works with small profit margins. The high cost of many commercial biosensors would not make them an economically viable alternative.
– Many commercial biosensor companies have targeted the relatively "money rich" life sciences and drug discovery customers and that is where their application knowledge is based. They have focused on the R&D values whilst ignoring the concepts required for routine testing that is the backbone of the food sector.
– Some commercial biosensor tests show no advantage over their counterparts, e.g. the biosensor method may use the same sample preparation so there are no real time/cost savings.
– Biosensor applications on individual platforms have not reached a critical mass. The sector cannot retire the counterpart technology because there are still too few tests available on individual commercial biosensors.
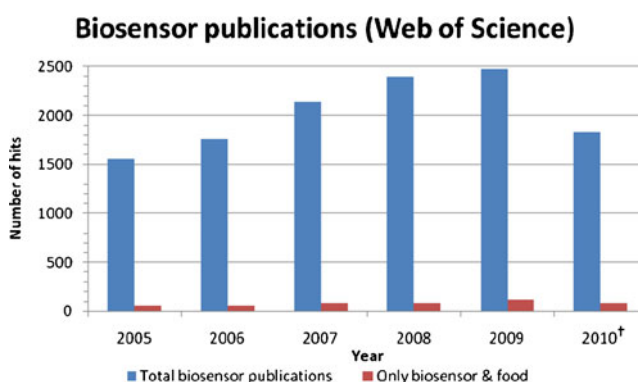

Fig. 1 Web of Science search for specific biosensor publications. *Dagger* data incomplete owing to the date of the search

Biosensors in food contaminant analysis

This review will now focus on the application of biosensors within a specific area of food analysis: the detection of contaminants. Food contamination can originate from a number of different sources, such as the improper use of veterinary drugs and pesticides, the formation of phytotoxins and marine toxins, bacterial contamination and the creation of chemicals during processing techniques. Analysis of food for contaminants is not only vital for our protection but it also aids the global trade process by helping to identify and limit the trade in contaminated produce [14]. Contaminants such as pathogens, toxins, pesticides and veterinary drug residues all have the potential to have serious health implications in terms of causing illness [27] or by limiting the efficacy of medicines through developed drug resistance [28].

The use of biosensors in food contaminant analysis must be placed in perspective. For toxins, pesticide and veterinary drug residues, there are physicochemical methods, such as liquid chromatography–tandem mass spectrometry (LC-MS/MS), which by European law must be used to confirm the presence of these compounds. These physicochemical methods tend to be expensive, complicated to perform and time-consuming. Biosensor methods can be used as a rapid screening tool so that only the small numbers of suspect samples are forwarded for confirmation, therefore greatly reducing the cost and time of analysis.

There are many review articles related to the use of biosensors to monitor ligand and receptor interactions, such as that by Rich and Myszka [12], which provide excellent guidelines for developers to help them distinguish between experimental artefacts and actual binding events, thus helping to improve the quality of biosensor publications. No such guidelines are available for the researcher who is using biosensors for food contaminant analysis. Most biosensor methods for food contaminant analysis are based on concentration analysis and use report points for end point measurements. In essence, an end point signal is obtained for different known concentrations of the analyte (often in the matrix of interest). A calibration curve is then plotted and unknown samples can be read against this calibration curve. The following are important for defining if a biosensor method for food contaminant analysis is fit for purpose:

– Proper fit for the calibration curve. The calibration curve needs to be the correct fit and not forced because of poor selection of calibrants.
– Sufficient dynamic range or resolution. The signal variation between concentrations is sufficient so that a small change in signal does not lead to a large change in concentration.
– Validation work outlined in legislation such as Commission Decision 2002/657/EC [29].

Sample preparation and matrix interference

An important consideration when using biosensors for food contaminant analysis is the impact of matrix components and ways to overcome this potential interference. In general, no matter which biosensor is used, some degree of sample preparation will be required. Sample preparation forms a vital role in food contaminant analysis, and comprehensive reviews are available, such as those by Kinsella et al. [30] and Novakova and Vlckova [31]. Sample preparation is often the bottleneck in analysis time [32]. Conventional approaches remain highly labour intensive and time consuming [31], whereas users require them to be as quick and cheap as possible with minimum steps, thus reducing analysis time and sources of error [32]. Sample preparation is matrix- and analyte-dependent [32] and its main aim is to isolate and preconcentrate the compound of interest whilst removing matrix interferences, such as fats, proteins, sugars and enzymes, that can affect the detection system [30, 31]. Not all transducers will suffer the same matrix interference issues. Typical sample preparations include simple liquid extraction, liquid–liquid extraction (use of solvents to repartition the analyte), solid-phase extraction (purification using sorbent materials), extraction with an organic solvent in the presence of salts followed by dispersive solid-phase extraction, and ultrafiltration [30, 31]. Magnetic immunoparticles may also be used to physically isolate and preconcentrate the analyte of interest, e.g. for the detection of pathogens [33]. No matter which extraction method is used, the analyte must be stable during all steps otherwise there will be an underestimation of the analyte concentration [31]. Minimal sample preparation techniques, e.g. extraction in aqueous buffer systems, are good at removing non-polar matrix components such as lipids, but other components that can interfere with the analysis will remain [30].

Another concern with crude sample preparations is that they may not facilitate the detection of the analyte at the concentration of interest, e.g. the biosensor method may not be able to detect a banned substance at its minimum required performance limit when extraction into aqueous buffer is employed. Not only can parent drugs and/or metabolites be extracted in aqueous systems, organic solvents can also be used that can allow sample concentration steps [30] following extraction, solvent evaporation and reconstitution back into aqueous buffers. For drug residues, pesticides and marine toxin analysis, acetonitrile is often used for liquid–liquid extraction owing to good yield of analytes with low levels of coextractants [30]. It is also useful in denaturing proteins [30]. Disadvantages include the environmental and safety implications of using toxic solvents. Many organic solvents are also incompatible for the extraction of hydrophilic compounds [31]. For the extraction of analytes that have become conjugated, enzymatic or chemical hydrolysis

will be required to liberate the compound before extraction [30]. When multiresidue extraction is being considered, there is always a compromise between recovery and purity [30]. The authors suggest that one should employ the least complicated technique and the minimal number of steps that will still allow the detection of the chosen analyte in the matrix at the required concentration of interest. For this reason, sample preparation needs to be tailored for the individual transducer and required detection level of the analyte.

Labelled versus unlabelled approaches in optical biosensors

It would be wrong to suggest that the use of labelled binding proteins can overcome matrix interference. In unlabelled systems, such as those using surface plasmon resonance (SPR), it is sometimes possible to see the binding of matrix components at the transducer as an unwanted signal that overpowers the specific binding that is of interest. In labelled systems, unwanted binding may still occur but, since the matrix component is not labelled, it would appear that there is no matrix effect. In reality all that can be said is that the binding of the matrix to the transducer has not produced a signal but may block the specific interaction with labelled binding protein, thus reducing sensitivity.

There are advantages and disadvantages in the use of fluorescently labelled binding proteins (antibodies and receptors) and aptamers over unlabelled equivalents:

– Advantages include the ability to improve the sensitivity of the test by choosing a dye with an intense signal and/or changing the ratio of dye to binding protein. A larger ratio should create an improvement in the relative sensitivity since the signal from the binding of a smaller amount of antibody will be increased.
– Disadvantages include an additional labelling step that needs to be well characterised to provide a reproducible result using different batches of labelled binding protein. Contamination may also become a problem. In certain samples, such as bile, it may not be possible to use labelled binding proteins since bile itself contains compounds that will fluoresce.

Portable biosensors for food contaminant analysis

Portability is an attribute that has been widely sought by the biosensor community as a whole. Fernandez et al. [34, 35] pointed out that the advances in microfabrication technology and improvements in signal optimisation to allow the resolution power to match that of larger laboratory-based biosensors have made this a reality. Indeed, it has been noted that the use of portable biosensors has the potential to revolutionise medical diagnostics and environmental

monitoring by allowing point-of-care and on-site testing [36]. Portable biosensors have also been applied to the analysis of food contaminants. Examples include the detection of antibiotic residues in milk using a portable optical (SPR) biosensor [34, 35], bacterial detection in dairy products using a portable electrochemical (conductometric) biosensor [37], detection of toxins in shellfish using a portable optical (SPR) biosensor [38] and the numerous food safety applications of the optical (fluorescence) array biosensor of the US Naval Research Laboratory (NRL) [39, 40].

There is a common limitation to the use of portable biosensors, namely the source of power. True on-site testing would require either the use of a dedicated battery or power delivered via a laptop connection, both of which will limit the working time of the biosensor depending on its power consumption. Another limitation, specific for food contaminant analysis, is the necessity of sample preparation. Portable biosensors are ideal for the analysis of liquid samples such as milk, water and fruit juices for contaminants that have reasonably high permitted levels (e.g. 100 ng/ml for certain drug residues), but when more complicated sample preparation is required, e.g. the homogenisation of meat samples or the requirement of enrichment steps or other forms of sample concentration, then even the most portable biosensor will be restricted by the preanalysis sample preparation which must be performed in a laboratory.

Commercial biosensors of note

Few commercial biosensor platforms have been developed which are targeted specifically at food analysis. This is not to say that non-food-orientated biosensors cannot be used for food analysis, but simply means that they may not have been designed with the specific requirements of food contaminant analysis in mind. Two platforms of note are the Biacore Q biosensor and the HLAB-2020 biosensor.

The Biacore Q biosensor The Biacore Q biosensor [41], produced by GE Healthcare, was developed for laboratory-based vitamin and food contaminant analysis using both reagents supplied in a kit format as well as providing users with the flexibility to develop tests using their own reagents, such as antibodies and receptors, which are natural binding partners located on cell structures, or aptamers, which are RNA or single-stranded DNA strands that bind to their target with high specificity and sensitivity by virtue of their three-dimensional shape. Aptamers are produced and selected using an approach of systematic evolution of ligands by exponential enrichment (SELEX) [42]. The Biacore Q biosensor is a label-free SPR biosensor that is capable of running only one channel of its four-channel configuration at a time. The system is fully automated, allowing the operator to leave after the assay has been started and providing unattended

analysis of up to 192 samples (if the user manually mixes the antibody with the sample). Historically, there are numerous food contaminant publications by numerous sources covering pesticide, drug residue, and toxin detection in various matrices. Publications on pathogen detection using the Biacore Q biosensor are relatively scarce, probably owing to limiting factors in the use of the SPR signal when working with large molecules, such as bacteria, that extend beyond the instrument's usual field of detection.

The HLAB-2020 biosensor The HLAB-2020 biosensor, produced by Hanson Technologies, is available for detection of *Escherichia coli* 0157:H7 and food allergens [43]. This is a commercialisation of the NRL's array biosensor. The NRL's array biosensor is an optical biosensor that was developed for on-site screening of biological hazards. A good review of its function and applications is provided by Taitt et al. [39] and Ligler et al. [40]. Briefly, it was designed to be portable and to analyse multiple samples for multiple analytes using six channels (in the automated version). It can be used in a strip or spot formation. It requires the use of primary or secondary fluorescently labelled binding proteins (antibodies, receptors) and aptamers. Capture molecules are immobilised in an array format on an optical waveguide. Sandwich or competitive assays can then be developed when samples and binding proteins are placed into one of two removable six-chamber reservoirs for introduction into the instrument. The fluorescence of any captured labelled binding protein can then be detected using a CCD camera fitted with appropriate filters. On completion of the interaction cycle, the location and intensity of these fluorescent strips or spots will ultimately inform the user as to what contaminant is present and at what concentration [39, 40].

Pathogens

There are five main bacterial pathogens (Table 3) which collectively account for most food-borne illnesses [44]. Their importance is based on the severity of infection and/or the number of outbreaks [44, 45]. Published biosensor articles have not been limited to these and cover many others, such as *Staphylococcus* [46], *Bacillus* [47, 48] and *Shigella* [49]. Commission Regulation (EC) No 2073/2005 [50] governs the microbial criteria for foodstuffs and covers permitted limits and reference methods. Bacterial pathogens are measured in terms of colony forming units (CFU) per millilitre or per gram. The legislation allows alternative analytical methods as long as they have been validated against the reference method. If the test is proprietary, then it also needs to be certified by a third party using internationally accepted protocols [50].

Table 3 Major food-borne bacterial pathogens

Pathogen	Example limits in food
Salmonella	Absent within a set weight of foodstuff during shelf life
Campylobacter	
Escherichia coli O157	50 CFU/g minced meat during processing (value set for *E. coli*)
	1,000 CFU/g ready-to-eat fruit and vegetables (value set for *E. coli*)
Listeria monocytogenes	100 CFU/g ready-to-eat foods during their shelf life
Clostridium perfringens	

CFU colony forming unit

According to a European Union report released in 2010 [45], salmonella was the most frequently reported cause of food-borne bacterial outbreaks in the European Union during 2008. In general, the total number of reported pathogenic bacterial outbreaks was similar to that for 2007.

The widely accepted gold standards for monitoring pathogens in food are microbiological methods [51–54]. Microbiological methods generally consist of an enrichment stage (preenrichment and/or selective enrichment), culturing in selective or differential agar plates to isolate cultures, followed by phenotype analysis or metabolic fingerprint analysis to confirm the result [49, 53]. Numerous articles have reported the problem of microbiological methods as being the time taken to obtain a result. Estimates range from 2 to 10 days depending on whether one is concerned with the first results or confirmation [47, 49, 55–57]. In many cases foodstuffs are delayed in shipment until the results of the tests are available [58]. This can have an impact on the shelf life of fresh produce. With the level of bacterial infections and the delay in the produce reaching the market, there is a clear need for a faster method. Several different technologies have emerged, namely polymerase chain reaction (PCR) methods, enzyme-linked immunosorbent assays (ELISA), biosensors, ultrasound and flow cytometry [52, 59, 60].

Biosensors for pathogen detection

Since 2007 there have been articles related to food-borne pathogen detection that cover many of the transducers listed in Table 1. e.g. electrochemical (amperometric) [61], optical (luminescence [33] and SPR [62]), mass/acoustic (piezoelectric) [60] and mass (cantilever) [63] transducers, although some are very much proof-of-concept biosensor that require more work to prove their use with actual food samples. Some biosensor articles have described sample preparations that have attempted to remove the time-

consuming enrichment or culturing steps by using magnetic particles. Antibodies that are specific for membrane antigens on bacteria have been immobilised to these magnetic particles, which can then be mixed with the sample. The samples can then be passed through a magnetic field, which will attract these particles and thus concentrate and purify the bacteria from the whole sample before biosensor analysis [33, 57, 64]. Most articles describe methods that attempt to remove the need for culturing and characterisation.

Antibody sandwich approach

One such approach is the use of sandwich assays, whereby antibodies specific for types of bacteria interact with the bacteria in the sample and a secondary antibody conjugated to a signal-generating moiety, either by itself or with a substrate, can be used to produce a concentration-dependent signal. Lin et al. [56] and Pal et al. [47] achieved this using electrochemical biosensors, whereas Valadez et al. [53] used fibre-optic biosensors that detected fluorescence. Lin et al. [56] described a rapid non-flow electrochemical (amperometric) biosensor method utilising a horseradish peroxidase (HRP)-labelled secondary antibody and HRP substrate to generate the signal that could detect E. coli 0157:H7 within 1 h after receiving a milk sample, but had a linear range between 5×10^3 and 5×10^5 CFU/ml with a limit of detection (LOD) of 5×10^3 CFU/ml, indicating that more work was required on sample preparation to obtain the necessary sensitivity. Pal et al. [47] described a sandwich-based capillary flow electrochemical (conductometric) biosensor for the detection of Bacillus cereus in alfalfa sprouts, strawberries, lettuce, tomatoes, fried rice and cooked corn after a simple sample preparation of homogenisation in a stomacher, filtration and dilution. They made novel use of polyaniline-tagged antibodies for binding to the pathogen before further antibody capture on the surface. The conductive polyaniline formed a bridge between electrodes, generating a concentration-dependent signal. The LODs were 62.7, 40.7, 35.3, 88.4, 72.6 and 58.0 CFU/ml for the six food types, respectively. They observed non-linearity in the concentration-related signal and advised that although the method could not be used to enumerate the number of cells, owing to its rapid detection time (6 min), it could be used to identify contaminated food samples.

Valadez et al. [53] achieved a LOD of 10^2 CFU/ml for Salmonella enterica in egg and chicken after 6 h enrichment and 1.5 h on their flow-based, optical (fluorescence), fibre-optic biosensor when utilising a fluorescently labelled secondary antibody. Ohk et al. [65] described an alternative, optical (fluorescence) based, fibre-optic approach in which they used a capture antibody and a fluorescently labelled aptamer to sandwich Listeria monocytogenes with a LOD of 10^2 CFU/25 g in ready-to-eat meat samples following homogenisation in a stomacher. An enrichment period of 18 h and 4 h biosensor detection is required. This is quicker than the microbiological method, but the biosensor methods described in the next sections are faster.

Biosensors utilising polymerase chain reaction

Several articles have been published showing biosensor methods that incorporate a PCR step [60, 61]. Chen et al. [60] immobilised an oligonucleotide specific for a gene of E. coli O157:H7 onto a mass/acoustic (piezoelectric) transducer in their flow-based biosensor. Apple juice, milk and ground beef samples underwent several centrifugation processes to isolate the bacteria, then genomic DNA was extracted and the specific gene was amplified by PCR using synthetic primers (including a tag sequence). The amplicons were denatured and allowed to hybridise with the immobilised oligonucleotide before a gold-nanoparticle-labelled secondary oligonucleotide that was complementary to the tag was introduced. This gold label amplified the signal, providing a LOD of 5.3×10^2 CFU/ml without enrichment. Sample preparation and analysis took 3 h. Poehlmann et al. [61] used an electrochemical (amperometric) portable flow-based biosensor for the detection of E. coli in meat juice. They immobilised a specific nucleotide sequence to capture the amplified 16S ribosomal RNA that had been isolated from the bacteria using an RNeasy Mini kit. They then used nucleotide conjugated esterase 2 as a reporter enzyme specific for bacterial 16S ribosomal RNA to complete the sandwich. The addition of substrate caused a concentration-dependent signal that was linear between 10^2 and 10^7 CFU/ml, giving a LOD of 500 CFU/ml in buffer, although the reported sample preparation, for meat juice, and the analysis time were much longer than those described by Chen et al. [60].

Novel biological components

Several articles have emerged showing the use of novel biological components for the detection of bacterial pathogens. Banerjee and Bhunia [48] used mammalian cells (Ped-2E9), with specific sensitivities for pathogens in a non-flow portable optical (absorbance) biosensor that assessed cytotoxicity by measuring the colour change caused by alkaline phosphatase activity. They detected L. monocytogenes in ready-to-eat meats and rice at 10^3–10^4 CFU/ml following enrichment for 4–6 h. Using the mammalian cells, they distinguished pathogenic from non-pathogenic bacteria. The use of mammalian cells has greatly reduced the time of analysis in comparison with the time required for the traditional microbiological methods and allows the distinction between pathogenic and non-pathogenic bacteria, but it has to overcome similar challenges, namely problems associated with keeping the cell line alive and free from contamination.

Shabani et al. [52] used bacteriophages for the direct detection of *E. coli* in an array-format electrochemical (conductometric) assay. In their proof-of-concept work they achieved a LOD of 10^4 CFU/ml. Using bacteriophages has several advantages in that they are ubiquitous in nature, show highly specific sensitivity for individual bacteria and are cheaper to produce/use than antibodies [52]. The fact that they are ubiquitous in nature may also be a challenge.

Cheng et al. [33] and Luo et al. [66] both described experiments that measure bioluminescence caused by isolated bacterial ATP in milk or meat juice. Cheng et al. detected *E. coli* in milk at 20 CFU/ml using immunoparticles to specifically capture and isolate the bacteria before bacterial ATP was released in the presence of luciferin–luciferase to produce the optical (luminescence) signal. Luo et al. detected *E. coli* and *Staphylococcus aureus* in beef juice at 10^3 CFU/ml levels while employing a mechanical separation of non-bacterial ATP in their disposable optical (luminescence) biosensor. These ATP-based approaches can distinguish between viable and non-viable cells, give results similar to those of traditional plate count tests, do not require culturing steps and show potential for online monitoring [33, 66], although sample preparation is critical in that all living organisms produce ATP and so the specific bacterial ATP needs to be carefully isolated.

Advances in biosensors for pathogen detection

This section highlights advances in pathogen detection by biosensors in terms of combining both functional and structural components to make the biosensor more compact and portable as well as advances in shifting from single analyte detection to improving multiplexing applications using arrays and multichannel instruments. Meta et al. [67] showed a proof-of-concept for the detection of *S. enterica* with a linear range between 10^2 and 10^8 CFU/ml using an integrated electrochemical (both amperometric and conductometric) biosensor in which the structural components of the system were also the functional components. Magnetic immunoparticles were used to capture the bacteria and an enzyme, pyrroloquinoline quinone dependent glucose dehydrogenase, incorporated in the biosensor structure facilitated electrochemical signal amplification and detection. A degree of sample preparation had also been incorporated into the low-cost and potentially portable device.

Multiplexing techniques are also advancing rapidly. Shriver-Lake et al. [51] described an experiment to detect *Salmonella typhimurium* in milk (LOD 5×10^6 CFU/ml) and apple juice (LOD 5×10^5 CFU/ml) in 45 min with the optical (fluorescence)-based portable NRL array biosensor. They described a sandwich-based multiplexing analysis using different fluorescent dyes. Samples are neutralised and diluted before flowing over the microarray-based surface, where the analyte is captured by an immobilised antibody. A fluorescently labelled secondary antibody is then introduced to generate a signal. The authors noted that the experiment they performed indicated that enrichment steps were required to reach the required sensitivity.

Karsunke et al. [68] described a proof-of-concept, six-flow-channel, multiplexing optical (luminescence) biosensor in a disposable microarray format, which they used to detect *E. coli* O157:H7, *S. typhimurium* and *Legionella pneumophila*, in buffer, at 1.8×10^4, 2.0×10^7 and 7.9×10^4 cells per millilitre, respectively. They immobilised immunospecific antibodies in a microarray format. Other immunospecific antibodies labelled with HRP were then added. The signals produced, on each spot, by HRP substrate reactions were detected by a CCD camera.

Several multiplexing biosensors have been described that utilise PCR [49, 69]. Koets et al. [69] focused on a giant magnetoresistance biosensor for detecting *E. coli* or four different antibiotic-resistant genes in salmonella using a double-tagged PCR amplification step, one oligonucleotide tagged with fluorescein and the other biotinylated. Magnetic particles coated with streptavidin were mixed with the samples, to concentrate the analyte, and passed over the surface of the giant magnetoresistance sensor that had antifluorescein antibodies immobilised. Unlike in other examples described earlier, the intact PCR product interacts with the surface, which involved the denaturing of the PCR product before introduction to the surface. Koets et al. explained that by the use of specific immobilised antibodies and tags, the instrument could be easily multiplexed. Bai et al. [49] described work that facilitates the detection of 11 food-borne pathogens, in buffer and pork meat, using a microarray approach with biospecific DNA probes immobilised on a sensor surface. The PCR products were denatured and allowed to hybridise with the immobilised probes; thus, the primer region on the biotinylated PCR products were able to bind with their relevant probes. Antibiotin IgG conjugated to HRP was then passed over the surface to complete the sandwich, followed by substrate, which facilitated the detection of the PCR product as a concentration-dependent colour change on the surface of the optical (absorbance) biosensor. Bai et al. suggested that real samples will require a long culturing step, 18–24 h, before PCR amplification is performed, but this is surely balanced by the fact that at least 11 pathogens can be detected in one sample run.

Future for biosensors for pathogen detection

It is clear that sample preparation is still a major issue affecting the speed of attainment of results. In an ideal

world, the total sample preparation time should be sufficient to allow preparation, detection and reporting of multiple samples in 1 day. The advancements in multiplexing and the moves towards portable and integrated instrumentation should greatly improve the profile of biosensors. One thing that has been absent from the articles is information on the automation potential of the systems developed. If the amount of human involvement in the chain could be reduced, then the usability of the instrument and reproducibility of the results could make them more attractive in routine testing.

Toxins

The consequences of consuming food contaminated with toxins range from mild gastrointestinal problems to death depending on the toxin and its concentration [39]. Toxins with food implications cover a large variety of molecules and so can be grouped under the following subheadings: plant/bacterial toxins, mycotoxins and marine toxins. This review focuses on publications that describe toxin detection in food matrices and not those describing detection in buffer systems.

Toxins are produced by living organisms and can range in size from a molecular weight of a few hundred to several hundred thousand daltons [39]. For larger toxins, it is possible to use non-competitive [20] and sandwich [70] based biosensor methods; lower molecular weight toxins require inhibition/competition-based methods [71].

Plant/bacterial toxins

Plant toxins such as ricin and bacterial toxins such as botulinum neurotoxins have been increasingly studied owing to the threat of their use in a terrorist attack on drinking water and food [70, 72]. Other bacterial toxins such as staphylococcal enterotoxins are of concern owing to health concerns associated with consuming contaminated food [73].

Biosensors for plant/bacterial toxin detection

As in pathogen analysis, for many years, biosensors showing single analyte detection were the "norm" for plant/bacterial toxin determination and in some instances their development has progressed in parallel with the advances made by other research groups. Maraldo and Mutharasan [74] showed the detection of staphylococcal enterotoxin B in apple juice (LOD 100 fg/ml) and milk (LOD 10 fg/ml) after sample dilution. Antibodies were immobilised onto mass/acoustic (microcantilever) transducers before samples were allowed to flow over the surface. Tsai and Li [73] developed a non-flow optical (SPR)-based biosensor for the detection of staphylococcal enterotoxin A in

milk. They immobilised antibodies to the surface and achieved a working range of 0.1–1.0 μg/g with untreated milk samples.

Advances in biosensors for plant/bacterial toxin detection

This section highlights advances in plant/bacterial toxin detection by biosensors in terms of multiplexing approaches, and attempts to minimise/remove sample preparation steps. The NRL array biosensor, as described previously, has been used to detect many toxins in food matrices [39], but these are beyond the scope of this review because of the date of publication, although the multiplexing blind trial by Shriver-Lake et al. [51] also included the detection of staphylococcal enterotoxin B in water, apple juice (LOD 100 pg/ml) and milk (LOD 1 ng/ml). Milk was a problem matrix, reducing the sensitivity of the test by one order of magnitude compared with buffer and apple juice.

Another multiplexing approach was described by Pauly et al. [72], whereby ricin, abrin, botulinum neurotoxins type A and type B and staphylococcal enterotoxin B are simultaneously detected in milk, baby food, yoghurt, iced coffee and carrot juice using a suspension-bead-array-based optical (fluorescence) biosensor. Specific antibodies for the analytes were immobilised to magnetic beads that were differentially fluorescently coloured. These were then mixed with homogenised samples, then magnetically isolated, concentrated and washed before a secondary fluorescently labelled antibody was introduced. They were then able to identify the bead, thus the analyte, and quantify its concentration in 50 μl of sample. They observed matrix interference that reduced the sensitivity to low nanogram per litre levels, but claim that the method is still applicable because the sensitivity was two to three orders of magnitude below the oral LD_{50}.

Yang et al. [75] described a novel portable and potential multiplexing electrochemical (conductometric) approach for the detection of staphylococcal enterotoxin B in baby food which they claim can be free from sample preparation. Their biosensor consisted of a network of single-walled carbon nanotubes, immobilised with antibodies, forming a biological semiconductor layer that shows a specific resistance. On binding analyte in the sample, the network connections are disrupted, changing the resistance in proportion to the concentration of the analyte bound to the antibodies. They claim that the fabrication of the sensor with 16 individually functionalised biological semiconductor regions will allow multiplex analysis of multiple analytes.

Future for biosensors for plant and bacterial toxins

Biosensors for plant and bacterial toxins will definitely become more portable in the future, driven by concerns of

deliberate contamination of the food system. Multiplexing will be a necessity and sample preparation will need to be rapid and simple, requiring few steps and capable of extracting multiple toxins.

Mycotoxins

Mycotoxins are a diverse group of secondary metabolites produced by certain fungi [76, 77] and are prevalent in wet and humid conditions [78, 79]. They can be carcinogenic, immunotoxic, nephrotoxic, teratogenic or act as endocrine disruptors [77, 80]. Mycotoxins have been associated with a range of foodstuffs, including cereals, nuts, fruits, spices and milk, and biosensor methods have been developed for their detection in these matrices (Table 4). Maximum residue limits (MRLs) have been established for many mycotoxins in various foodstuffs, although within Europe these have not been harmonised into one document, so several documents have to be consulted: Commission Regulation (EC) 1126/2007 [81]; Commission Regulation (EU) No 165/2010 [82]. Alternative screening methods such as ELISA and lateral flow devices have also been employed for mycotoxin detection [71]. Confirmatory methods are moving towards multiple mycotoxin detection using LC-MS/MS [83, 84].

Biosensors for mycotoxin detection

Paniel et al. [79] provided a useful example of single analyte analysis with the detection of aflatoxin M1 in milk

Table 4 Selected matrices with biosensor methods for mycotoxin analysis

Mycotoxin	Matrix	Transducer and biological component
Aflatoxin B1	Spice	Electrochemical (amperometric) antibody [78]
	Olive oil	Electrochemical (amperometric) enzyme [158]
Aflatoxin M1	Milk	Optical (fluorescence) antibody [85]
		Electrochemical (amperometric) antibody/enzyme [79]
Ochratoxin A	Wheat	Electrochemiluminescent aptamer [86]
	Beer, coffee	Electrochemical (amperometric) [159]
Zearalenone	Corn silage	Electrochemical (amperometric) antibody [160]
Trichothecenes		
Deoxynivalenol	Cereals, baby food	Optical (SPR) antibody [71]
T2 and HT-2	Cereals, baby food	Optical (SPR) antibody [83]

(European MRL 0.05 ng/ml, provided in Commission Regulation (EU) No 165/2010 [82]). They demonstrated an electrochemical (amperometric) method using magnetic nanoparticles, immobilised with aflatoxin M1 antibodies, to separate and concentrate the sample with minimal preparation. Following wash steps, a competition is set up for the antibody between aflatoxin M1 in the sample and a fixed concentration of HRP-labelled aflatoxin M1. After washing, the beads are introduced to the screen-printed electrodes and a mediator, 5-methylphenazinium methyl sulfate, is added. The intensity of the signal produced is related to the amount of bound HRP-labelled aflatoxin M1. With this method they achieved a LOD of 0.01 ng/ml (five times lower than the MRL).

Wang et al. [85] demonstrated an optical (fluorescence) method for the detection of aflatoxin M1 in milk using long-range surface-plasmon-enhanced fluorescence spectroscopy. This method is based on the principle that a fluorophore, in close proximity to the sensor surface, will be excited by surface plasmons and fluoresce. Competition was set up for the fluorophore-labelled aflatoxin M1 antibody between immobilised aflatoxin M1–bovine serum albumin and aflatoxin M1 in the sample. By simply centrifuging the milk, they achieved a LOD of 0.6 pg/ml, raising the question of how sensitive the assay needs to be. Wang et al. [85] obtained a LOD almost 17 times lower than Paniel et al. [79], but both groups obtained LODs lower than the MRL, so both are fit for purpose. Preference for routine testing will depend on the cost of equipment, ease of use and the analysis time (both methods use the same simple sample preparation).

Wang et al. [86] provided an example of a novel aptamer-based electrochemiluminescent biosensor for the detection of ochratoxin A in wheat whereby a single-stranded DNA sequence is immobilised and a complementary strand labelled with a luminol-based compound is then introduced. In the presence of hydrogen peroxide, this label produces an electrochemiluminescent signal proportional to the amount of luminol at the surface. This complementary strand also forms a complex with ochratoxin A, allowing the development of a displacement assay capable of detecting 7 pg ochratoxin A per millilitre in wheat samples using a simple extraction/filtration method.

Advances in biosensors for mycotoxin detection

This section highlights advances in mycotoxin detection by biosensors in terms of advances in applications that allow multiple sample types to be processed simultaneously without the need for matrix-matched calibration curves and improvements made in the time for analysis. Meneely et al. [71] described advances using an optical (SPR) biosensor for the detection of deoxynivalenol in wheat (LOD 57 μg/

kg), wheat products (LOD 9 μg/kg) and maize-based baby food (LOD 6 μg/kg). Following a sample preparation involving solvent extraction, evaporation and reconstitution, a flow-based competitive assay was constructed between the immobilised deoxynivalenol derivative and free analyte in the sample. Their method did not need matrix-matched calibration curves, thus facilitating determination of multiple matrices within a run. They also claim to have made improvements in both sample preparation and the time of analysis compared with other biosensor methods.

Future for biosensors for mycotoxins

In general, it could be suggested that biosensor advancements in mycotoxin detection are moving towards faster and less complicated sample preparations, analysis of multiple sample matrices in one run and simultaneous detection of families of mycotoxins. Proof-of-concept work by Mak et al. [76] suggests that the next step could be the multiplexed detection of compounds from different families within one run. In their work they detected mycotoxins from different families at 50 pg/ml in buffer using a portable giant magnetoresistance biosensor. Antibodies immobilised to the array captured the analyte from the sample and a secondary biotinylated antibody was then added. A concentration-dependent signal was then produced on the addition of streptavidin-labelled magnetic nanotags.

Marine toxins

Marine toxins cover several diverse groups of compounds with distinct structures [87]. Most are formed by microalgal species and dinoflagellates, and under certain ecological conditions these species can grow into very large colonies. The toxins can accumulate in filter-feeding bivalves such as shellfish [87]. Fusetani and Kem [88] provided a very useful report covering freshwater and marine toxins, whereas Dominguez et al. [89] and Campas et al. [90] focused on toxins with food safety concerns (Table 5). European MRLs have been established for some marine toxins: Regulation (EC) No 853/2004 [91]. The mouse bioassay is the most widely used test for marine toxins [87], but it has associated ethical concerns [87, 90, 92] and so alternative methods are being developed in an attempt to replace it. Chromatography-based methods such as high-performance liquid chromatography with fluorimetric detection and LC-MS/MS are available [92], but these are time-consuming and costly [90]. Other methods such as protein phosphatase inhibition assays, cytotoxicity assays and immunoassays have also shown promise for some groups of marine toxins [92].

Table 5 Marine toxins of concern in food safety (compiled from [88–90])

Toxin group	Example
Diarrhoeic shellfish poisoning toxins	Okadaic acid
Pectenotoxins	Pectenotoxins
Paralytic shellfish poisoning toxins	Saxitoxin
Amnesic shellfish poisoning toxins	Domoic acid
Yessotoxins	Yessotoxin
Neurologic shellfish poisoning toxins	Brevetoxins
Azaspiracid shellfish poisoning toxins	Azaspiracid
Palytoxins	Palytoxins
Ciguatera fish poisoning toxins	Ciguatoxins
Puffer fish poisoning toxins	Tetrodotoxin
Cyclic imines	Pinnatoxins
Microcystins	Microcystin-LR

Biosensors for marine toxin detection

An optical (SPR) biosensor method was developed by Fonfria et al. [93] for the detection of paralytic shellfish poisoning toxins in mussels, clams, cockles, scallops and oysters with LODs between 2 and 50 ng/ml (sufficient for 80 μg/100 g tissue) . They looked at various extraction methods and concluded that an ethanol extraction, evaporation and reconstitution in buffer suited their needs. By using antibodies in a competition-based format, with their method they detected some other paralytic shellfish poisoning toxins showing different cross-reactivity. On comparison with two accredited methods, they concluded that their method was suitable to screen for toxins even with its low recovery of approximately 60%.

Advances in biosensors for marine toxin detection

This section highlights advances in marine toxin detection by biosensors in terms of improvements in portability, multiplexing and simultaneous generic detection of whole families of marine toxins. A novel method was presented by Stevens et al. [38] whereby they developed a six-channel portable optical (SPR) biosensor with integrated fluidics for the detection of domoic acid, with a LOD around 3 μg/kg, in clams using methanol extraction and solid-phase-extraction sample cleanup. They utilised an antibody-based assay in both competition and displacement formats. Portability is a key desire because it allows detection at the shellfish bed, thus allowing samples to be tested before going to the expense of harvesting and returning to shore for analysis. A positive result at this point would require the catch to be disposed of, whereas detection at sea would allow the ship to relocate to another bed that shows acceptable toxin levels. Unfortunately, the sample preparation Stevens et al. used

included a solid-phase extraction which does not lend itself well to field analysis.

An interesting progression can be observed in development of biosensor methods for diarrhoeic shellfish poisoning toxins starting with Llamas et al. [94], who developed an optical (SPR) antibody-based competitive method for okadaic acid detection in mussel (LOD 126 ng/g) following homogenisation, methanol extraction, evaporation and reconstitution in buffer. They observed that their method was unable to detect other members of the group, and pointed out this detection would have been desirable. Stewart et al. [95] were then able to further this work using the same optical (SPR) biosensor and showed that, with considered selection of a monoclonal antibody, it was possible to develop the test to show multiplexed cross-reactivity for three compounds within the group (okadaic acid, dinophysistoxins 1 and 2) that mimicked the toxic profiles before fully validating the method in a matrix showing a working range of 31–74 μg/kg and a LOD of 31 μg/kg [96]. Having compared the method with liquid chromatography–mass spectrometry and in the belief that any replacement for the mouse bioassay for diarrhoeic shellfish poisoning toxins should be able to account for relative toxicities in the group, they postulated that their method could be used as a screening tool with only suspect samples being forwarded for confirmation [96].

Future for biosensors for marine toxins

It is clear that there is a desire to move away from the mouse bioassay, and biosensors could form a part of the transition. Botana et al. [87] suggested that the use of receptors which recognise toxicity would be a major advantage especially when used in conjunction with a confirmatory method. Marchesini et al. [97] are of like mind in that they have shown proof-of-concept in developing an antibody-based screening method for paralytic shellfish poisoning toxins that couples an optical (SPR) biosensor with a liquid chromatography–mass spectrometry procedure to allow preliminary screening, via a competition-based assay, and confirmation of toxin.

Pesticides

The term "pesticide" refers to a variety of products that are used to control pests and is often used as a synonym for plant protection products [98, 99]. Approximately 1,000 pesticide active ingredients are recognised worldwide [100]. Common subclasses of pesticides are insecticides, fungicides, herbicides, molluscicides, rodenticides, plant growth regulators and bird/animal repellents [98]. This review will cover chemical pesticides, which can be categorised into organophosphate pesticides, carbamate pesticides, organochlorine insecticides or pyrethroid pesticides [101].

Pesticides have been used for over 60 years to protect crops, by preventing diseases and infestations, and maintain the yield and quality necessary to meet consumer and government expectations [98, 99]. Within Europe and the USA, pesticides can only be used with prior authorisation [99, 102]. Pesticide residues pose a health risk [103–105], and as such their presence in foodstuffs has to be monitored. According to a European Union report [106], more than 70,000 samples, covering approximately 200 food types, were tested across Europe in 2008 for pesticides and only 3.5% were above their MRL. Council Regulation (EC) No 396/2005 [107] governs MRLs in Europe and the Environmental Protection Agency sets the tolerances in the USA [102]. Confirmation methods for pesticide residues are based on gas or liquid chromatography with a selective element detector [104, 108, 109], but these tend to be expensive, complicated to perform and time-consuming [104, 108]. To reduce the number of samples requiring confirmation, screening tests can be applied whereby only samples deemed to be positive are forwarded for confirmation [110]. There are many screening methods that have applications for pesticides: near-infrared reflectance spectroscopy [111], ELISA [112], fluorescence-linked immunosorbent assay [113], lateral flow devices [114, 115], high-performance thin-layer chromatography–enzyme inhibition [116], and biosensors [110] are a few examples.

Traditional acetylcholinesterase biosensors for pesticide analysis

Many of the biosensor methods developed for the detection of pesticides are based on the action of the enzyme acetylcholinesterase (AChE). The action of this enzyme (Fig. 2) is inhibited by pesticides [117–119]. Pesticides such as organophosphates and carbamates bind to a serine moiety within the active site of the enzyme, thus preventing the deacetylation of acetylcholine [108, 120]. The problems with this approach are that other compounds such as heavy metals and detergents also selectively inhibit the enzyme, the enzyme is unstable outside its natural environment and each pesticide has different affinities for the enzyme [103].
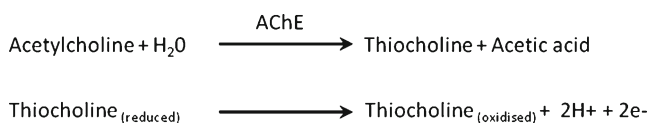
$$Acetylcholine + H_2O \xrightarrow{AChE} Thiocholine + Acetic\ acid$$

$$Thiocholine_{(reduced)} \longrightarrow Thiocholine_{(oxidised)} + 2H^+ + 2e^-$$

Fig. 2 Mechanism of acetylcholinesterase (*AChE*)

Advances in acetylcholinesterase biosensors for pesticide analysis

Research has continued into the use of AChE biosensors, presumptively as a rapid screening tool. This section highlights advances in AChE-based biosensor detection of pesticides, the main focus being improved stability of AChE and increased sensitivity. Vamvakaki and Chaniotakis [121] detailed a cuvette-based experiment whereby they tested for organophosphates in drinking water with improved stability by encapsulating the enzyme within a liposome and detecting the change in acidity caused by production of acetic acid by monitoring changes in the fluorescence of pyranine on an optical (fluorescence) biosensor. They achieved a LOD of 10^{-10} M. Pyranine is pH-sensitive. AChE causes the conversion of acetylcholine to thiocholine and acetic acid, thus altering the pH. The action of the pesticide inhibits this reaction, thus interfering in the pH change and therefore affecting the fluorescence of pyranine.

Caetano and Machado [105] and Hildebrandt et al. [118] described electrochemical (amperometric) biosensors for the detection of pesticides in food samples based on the formation of products from the enzyme activity on the substrate. In each case a baseline activity reading for the biosensor is obtained and then sample is introduced for a period of time before the activity of the biosensor is reassessed. Any pesticide present in the sample will have caused a decrease in the activity of the biosensor. Caetano and Machado [105] immobilised AChE on a carbon paste electrode for the analysis of carbamates in liquidised tomato and achieved a working range of 5×10^{-5}–75×10^{-5} mol/l in buffer, and a LOD in tomato close to the permitted levels in Brazil, by measuring the decrease in enzyme activity on its substrate, acetylthiocholine iodide, after exposure to carbamates. The work of Caetano and Machado goes a step further and describes the detection of pesticides in tomato pulp, without any sample preparation, although they admit that pesticides will more likely be found on the skin. Hildebrandt et al. [118] detected organophosphates and carbamates in water, vegetable extracts and beverages at 2 μg/l using screen-printed electrodes with immobilised AChE and monitored the signal caused by the conversion of thiocholine to dithiocholine with their portable system.

Roepcke et al. [108] have proved that it is possible to increase the sensitivity of the mechanism for a particular pesticide by first pretreating it with an oxidase enzyme to convert the pesticide into its more inhibitory oxon analogue. They directly measured phosphorothionate pesticides in orange juice at 25 μg/l using an electrochemical (amperometric) biosensor.

Some authors have moved away from directly detecting the products of AChE activity. Kim and Kim [122] and Kim et al. [119] both described proof-of-concept use of a mass/

acoustic (piezoelectric) transducer to detect the binding of pesticides to immobilised AChE. Kim and Kim [122] achieved a LOD of 1×10^{-9} M for paraoxon-ethyl detection, whereas Kim et al. [119] detected organophosphates (LOD 1.55×10^{-8} M) and carbamates (LOD 1.30×10^{-9} M). Both studies increased the mass associated with this interaction using a different substrate, 3-indolyl acetate, which under the action of AChE produces a precipitate on the crystal surface, thus increasing the mass. In the presence of pesticide this precipitate formation is inhibited.

Lee et al. [120] described a bienzyme approach for the detection of organophosphates whereby the choline product from the AChE reaction in solution acts as a product for the immobilised choline oxidase enzyme in their electrochemical (amperometric) biosensor. They suggest this is useful because it shows potential for on-site use and allows the experiment to run at a lower potential, thus reducing the unwanted oxidation of interfering compounds that occurs at higher potentials. Sun and Wang [117] described an electrochemical (amperometric) method utilising chitosan to allow the immobilisation of more AChE on glassy carbon electrodes and Prussian blue to enhance electron transfer. They detected organophosphates between 2.5 and 15 ng/ml and showed enhanced sensitivity and improved stability.

Norouzi et al. [123] used gold nanoparticles and multi-walled carbon tubes to promote electron transfer and catalyse the electrooxidation of thiocholine in their electrochemical (amperometric) biosensor method for the detection of monocrotophos (LOD 10 nM). Their flow-based system used glassy carbon electrodes that had been modified with gold nanoparticles and multiwalled carbon nanotubes. The nanotubes contained chitosan to increase the immobilisation level and improve the stability of AChE.

Advances using other biosensor approaches

This section highlights advances in pesticide detection using non-AChE-based biosensors to improve the stability and shelf life of the biosensor as well as make improvements to on-site applications. Other enzyme-based biosensors have been investigated. Shim et al. [124]. and Vidal et al. [109] both described electrochemical (amperometric) biosensors using tyrosinase for pesticide detection. The later work by Shim et al. [124] immobilised the enzyme on glassy carbon electrodes and detected parathion (linear range 0.1–1 ppb) and carbaryl (linear range 0.01–10 ppb) by measuring the enzyme oxidation and electrochemical reduction of catechol. As with most AChE-based methods, a baseline activity reading was taken in the substrate and then sample was introduced before changes in activity were measured when the biosensor was reintroduced to the substrate. The application showed improved stability over AChE methods. Murthy et al. [125] developed an electrochemical (potentiometric)

biosensor using immobilised dehydrohalogenase to release chloride ions, from DDT and hexachlorocyclohexane in water samples following acidification of the water and then solvent extraction and resuspension in aqueous buffer. The chloride ions could then be detected by the ion-selective electrode. They observed improved stability and usability of the system and believed it could be used for on-site monitoring.

Work has also been performed using calf thymus DNA [126, 127]. Solanki et al. [126] showed the use of both electrochemical (amperometric) and optical (SPR) biosensors whereby a pesticide is complexed with the immobilised double-stranded DNA in their flow-based systems. They detected cypermethrin in water (LOD 0.0005 ppm). For the amperometric biosensor, application of a potential to the immobilised electrode resulted in oxidation of nitrogenous bases, e.g. guanine. The guanine oxidation current was observed to decrease with increasing concentrations of cypermethrin. Kaushik et al. [127] introduced nanoparticles to improve the immobilisation stability of the calf thymus DNA on their disposable electrochemical (amperometric) biosensor. They detect cypermethrin (linear range 0.0025–2 ppm) and permethrin (linear range 1–300 ppm).

A novel proof-of-concept was described by Buonasera et al. [110]. In their work they used a whole organism (*Chlamydomonas reinhardtii*) to detect several herbicides in water (LOD 10^{-10}–10^{-8} M) using both optical (fluorescence) and electrochemical (amperometric) techniques. The approach was based on inhibition of photosystem II within the organism. The herbicides block photosystem II by displacing a specific molecule from the D1 protein, which causes an interruption in electron flow and oxygen evolution whilst also directly changing the fluorescence properties of photosystem II. Unfortunately the system shows a weakness similar to that of AChE in that the inhibition is also not limited to herbicides. Buonasera et al. postulated that it would be possible to use genetically modified strains of the organism to attain the required specificity and that both transducers facilitate the development of easy, low-cost and fast multiplexing arrays for prescreening. This is still very much in its infancy and the authors acknowledge that when they introduce matrices they may have to overcome problems in sample preparation.

Future for biosensors for pesticides

Biosensors do play a role in pesticide analysis although they are generally used as a crude prescreen to reduce the overall cost of sample analysis by identifying suspect samples to be analysed further by confirmatory methods. There are far too many pesticides in use and under development that differ significantly in structure, preventing effective use of biospecific antibodies with existing transducer technology. The

use of enzymes such as AChE has prevailed simply because of their broad spectrum inhibition. Work has shown that it is possible to improve sensitivity towards weaker-affinity pesticides and the authors are sure the stability of immobilised AChE will only improve, providing better tools for remote analysis. It should be interesting to follow further work on the photosystem II approach, but the degree of uptake by the community will hinge on the extent of sample preparation that may be required. If the amount of sample preparation is considerable, then this would have to be weighed up against potential benefits (throughput, speed and cost) to determine if there are sufficient advantages for it to be practical.

Veterinary drug residues

Consumption of food containing veterinary drug residues can lead to the selection of resistant bacteria in the gut [128, 129], hence reducing the efficacy of drugs for fighting infection in humans [128, 130]. There is also an increased risk of drug sensitisation, and thus allergic reactions, and disruption of the natural ecological equilibrium [131]. For these reasons MRLs have been established for drug residues in food. Within Europe these are controlled by Council Regulation (EC) No 470/2009 [132] and Commission Regulation (EU) No 37/2010 [133], whereas Council Directive 96/23/EC [134] instructs member states on how to construct a national residue monitoring plan (surveillance scheme). In the USA, US Federal Government 21 CFR 556 [135] provides tolerances and US Federal Government 9 CFR 310 [136] provides guidance for the US National Residue Program "Blue Book" and results "Red Book" [137]. Regulations stipulate that physicochemical methods such as gas chromatography–mass spectrometry and LC-MS/MS are required to positively confirm the presence of a residue. As stated before, these physicochemical methods are expensive, time-consuming and require highly trained personnel and often elaborate sample preparations [138–140]. This is why many laboratories employ a two-step approach; a rapid, less expensive, screening procedure with only suspect samples being forwarded for confirmation [138, 140]. According to Jornet et al. [141], the speed of screening is not necessarily the most important thing. It is feasible to allow more time to reach the required sensitivity, e.g. in the case of a banned substance, whereby a faster method may not have the required LOD, allowing residues to pass undetected into the food chain. Many different types of food or animal by-products can be analysed to test for drug residues in food. Table 6 provides several recent examples.

Table 6 Recent examples of matrices for drug residue testing

Matrix	Residue	Mode of detection
Meat	Tetracycline group	Induction of bioluminescent whole cell [128]
Urine	Clenbuterol	Labelled residue competition for antibody (optical–fluorescent) [161]
Honey	Chloramphenicol	Antibody competition with nanoparticle enhancement (optical–SPR) [162]
	Sulfathiazole	Antibody competition (optical–fluorescent) [141]
Honey/milk	Tetracyclines	Receptor competition (optical–SPR) [163]
Milk	Anti-inflammatory drugs	Enzyme inhibition (electrochemical–amperometric) [164]
	Benzimidazole	Antibody competition (optical–SPR) [165]
	Penicillin	Enzyme activity enhanced by carbon nanotubes (electrochemical–amperometric) [131]

Advances in biosensors for veterinary drug residues

Veterinary drug residues, like the other contaminants, were traditionally detected by single-analyte systems. Over the years, there has been a shift in emphasis away from single-analyte tests and systems towards multianalyte detection [130]. Huet et al. [129] attribute this shift to increased sample throughput to Spinks [142] with her broad-specificity immunoassay for food contaminants.

Haasnoot et al. [143] showed the development of a single-analyte optical (SPR) method for the detection of the fluoroquinolone flumequine using an antibody and a flow-based biosensor, in broiler serum (LOD 15 ng/ml) and muscle (LOD 24 ng/ml). For the serum, sample preparation was by simple dilution, whereas homogenisation in buffer was utilised for muscle. This group of compounds is difficult to multiplex because flumequine does not possess the generic portion of the molecular structure common with the other fluoroquinolones. Marchesini et al. [144] developed what they call a dual biosensor allowing the detection of six fluoroquinolones, including flumequine. They used a multichannel optical (SPR) biosensor, Biacore 3000, in serial mode, to detect six fluoroquinolones in poultry muscle via a competition-based assay, following a simple buffer extraction. They mixed a flumequine-specific antibody with an antibody that cross-reacted with five other fluoroquinolones before injection over two immobilised flow cells. Interestingly, following a positive screen they were able to fractionate the suspect sample using gradient liquid chromatography before reintroducing it to the biosensor. Individual fluoroquinolones in the sample could then be analysed separately in a second run, allowing the operators to suggest which compounds should be investigated for confirmation analysis by liquid chromatography–mass spectrometry. Huet et al. [130] took fluoroquinolone detection to another level when they developed [130] and validated [129] a flow-based optical (SPR) competitive method that was capable of detecting 13 fluoroquinolones below their MRLs in egg (LOD 1 ng/ml), fish (LOD 1.5 ng/ml) and poultry meat (LOD <0.5 ng/ml), following buffer extraction. Instead of using serial flow channels, they worked with one channel and developed a very novel "biactive" antibody. They cleverly engineered their immunogenic conjugate with two different haptens, allowing them to produce a highly cross-reactive antibody.

Multiplexing via selective raising of antibodies can also be observed in the work of Thompson et al. [140] and Connolly et al. [139]. An antibody was originally produced with cross-reactivity to five nitroimidazoles and was utilised in a competitive assay on a flow-based optical (SPR) biosensor to detect the five nitroimidazoles in chicken muscle, at levels below 1 μg/kg, following a solvent extraction, evaporation and buffer reconstitution [139]. This was superseded by application of an antibody that showed cross-reactivity with seven of the parent nitroimidazoles and improved cross-reactivity to the metabolites. The method was applied to kidney, liver, eggs, serum and milk, with detection levels below 1 μg/kg, in the UK surveillance scheme [140].

Future for biosensors for drug residues

As stated in previous sections, not only has reagent development played a major role in application advancement, but instruments have also advanced to meet user needs. Although only in a buffer, Raz et al. [138] provided a good example of what to expect in the future. They presented a flow-based microarray optical (imaging SPR) application for the detection of seven compounds from four drug residue classes using antibodies (monoclonal antibodies against neomycin, gentamicin, chloramphenicol, sulfamethazine and dihydrostreptomycin, polyclonal antibodies against kanamycin and norfloxacin) in a competitive format. With careful selection of antibodies, this could lead to a powerful screening tool.

Adrian et al. [145] also presented multidrug family detection in milk, following sample dilution, using a flow-based wavelength-interrogated optical biosensor

with class-sensitive bioreceptors [antibodies for fluoro-quinolones and sulfonamides, TetR receptor/anti-TetR antibody for tetracyclines and rabbit serum albumin (RSA) receptor/anti-RSA antibody for β-lactams] as biological components. They claim to have detected 30 different antibiotics, although they based this claim on the cross-reactivity profile of their bioreceptors by ELISA. The article only shows the detection of one member from each of the four families, sulfonamides, fluoroquinolones, β-lactams and tetracyclines. They provided a proof-of-concept; it would be interesting to see the actual cross-reactivity profile using the biosensor technology since cross-reactivity profiles can differ significantly between ELISA and biosensors [146] and between different buffers and matrices [130, 140].

A limitation with both the imaging SPR and the wavelength-interrogated optical biosensor is that they both use a single channel to deliver reagents to all the biorecognition sites. This requires careful selection of antibodies/receptors based on cross-reactivity and a single sample preparation and regeneration for all analytes. This increases the likelihood that banned substances cannot be detected at the required sensitivities in the same experiment as substances with relatively higher MRLs. Prototype instruments with a limited array but multiple channels have also been developed. Campbell et al. [147] provided an example of one such instrument that could be a powerful tool allowing several different sample preparations to be processed simultaneously over a smaller biosensor array. They showed a proof-of-concept using a four-flow-channel parallel optical (SPR) biosensor, with four immobilising spots in each channel, for unattended multiplexed detection of high and low molecular weight compounds. The further development of such instruments will help speed up screening procedures and keep biosensors at the forefront of food safety.

Conclusion

Advances made recently in biosensor applications for the detection of food contaminants differ somewhat depending on the type of contaminant in question and the associated analytical requirements. However, it is clear from the literature reviewed that common needs are more often being addressed.

Analysis has been accelerated through the development of rapid sample preparation techniques, whereas integrated and automated systems allow the operator to start analysis and "walk away" while data are being generated. Improvements in automation save time and lower costs but also reduce the possibility of human error.

The simultaneous detection of more than one analyte in a sample has become a much sought after attribute of screening assays, and the production of generic recognition elements (antibodies, receptors, aptamers) goes some way to achieving this, usually within a family of compounds possessing similar chemical structure. However the increased use of array and multiple-channel systems adds a new dimension to multiplexing with the potential of detecting a number of unrelated pathogens, toxins, pesticides or drug residues or indeed a combination of contaminants from these different groups.

Portability is a feature that is required in the field or for point of-use-analysis (detection of contaminants as a result of terrorist activity or detection of marine toxins at sea) and is more valued when speed of analysis is the main issue. Leading the way on portability have been research groups such as Ligler et al. [40], Poehlmann et al. [61], Banerjee and Bhunia [48] and Mata et al. [67] with pathogen detection, Yang et al. [75], Mak et al. [76] and Stevens et al. [38] with toxin detection and Hildebrandt et al. [118] with pesticide detection. Further research into sample preparation techniques must be performed to help make more systems that are truly portable from the laboratory.

The ingenuity of researchers combined with advances in a range of biosensor technologies has allowed the needs of food analysts to be met and will continue to provide simple to use, inexpensive, multiplexed and portable systems that can be used to ensure the health and safety of consumers around the world.

The ultimate achievement for biosensor applications in food contaminant measurements would be the ability to directly couple the biosensor to a physicochemical technology, thus allowing rapid screening with direct confirmation of a positive sample. As yet this is still a dream because of the sample preparation demands of instruments such as those used in LC-MS/MS.

References

1. Nagel B, Dellweg H, Gierasch LM (1992) Pure Appl Chem 64:143–168
2. Thevenot DR, Toth K, Durst RA et al (1999) Pure Appl Chem 71:2333–2348
3. Renneberg R, Pfeiffer D, Lisdat F et al (2008) Adv Biochem Eng Biotechnol 109:1–18
4. Clark LC, Lyons C (1962) Ann N Y Acad Sci 102:29–45
5. Updike SJ, Hicks GP (1967) Nature 214:986–988
6. Cammann K (1977) Fresenius Z Anal Chem 287:1–9
7. Farre M, Kantiani L, Perez S et al (2009) Trends Anal Chem 28:170–185
8. Ma A (2009) A technical and commercial review of biosensor developments and the implementation of nanowire technologies
9. Bosch ME, Sanchez AJR, Rojas FS et al (2007) Comb Chem High Throughput Screen 10:413–432
10. Cock LS, Zetty Arenas AM, Aponte AA (2009) Chil J Agric Res 69:270–280
11. Luong JHT, Male KB, Glennon JD (2008) Biotechnol Adv 26:492–500

12. Rich RL, Myszka DG (2010) J Mol Recognit 23:1–64
13. Grieshaber D, MacKenzie R, Voeroes J et al (2008) Sensors 8:1400–1458
14. Viswanathan S, Radecka H, Radecki J (2009) Mon Chem 140:891–899
15. Jiang X, Li D, Xu X et al (2008) Biosens Bioelectron 23:1577–1587
16. Bosch ME, Sanchez AJR, Rojas FS et al (2007) Sensors 7:797–859
17. Orellana G, Haigh D (2008) Curr Anal Chem 4:273–295
18. Fan X, White IM, Shopoua SI et al (2008) Anal Chim Acta 620:8–26
19. Ermolaeva TN, Kalmykova EN, Shashkanova OY (2008) Russ J Gen Chem 78:2430–2444
20. Tark S, Das A, Sligar S et al (2010) Nanotechnology 21:435502
21. Wang L, Sipe DM, Xu Y et al (2008) J Microelectromech Syst 17:318–327
22. Ahmed MU, Hossain MM, Tamiya E (2008) Electroanalysis 20:616–626
23. Homola J (2008) Chem Rev 108:462–493
24. Ligler FS (2009) Anal Chem 81:519–526
25. http://apps.isiknowledge.com/summary.do?qid=17& product=WOS&SID=T1E8HHM8oeMjC492mb9&search_ mode=Refine. Accessed 3 Nov 2010
26. http://apps.isiknowledge.com/RAMore.do?product= WOS&search_mode=&SID=T1E8HHM8oeMjC492mb9&qid= 16&ra_mode=more&ra_name=PublicationYear&db_id=WOS &viewType=raMore. Accessed 3 Nov 2010
27. Kim SJ, Gobi KV, Iwasaka H et al (2007) Biosens Bioelectron 23:701–707
28. McGrath T, Baxter A, Ferguson J et al (2005) Anal Chim Acta 529:123–127
29. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C(2002) 3044)
30. Kinsella B, O'Mahony J, Malone E et al (2009) J Chromatogr A 1216:7977–8015
31. Novakova L, Vlckova H (2009) Anal Chim Acta 656:8–35
32. Ridgway K, Lalljie SPD, Smith RM (2007) J Chromatogr A 1153:36–53
33. Cheng Y, Liu Y, Huang J et al (2009) Talanta 77:1332–1336
34. Fernandez F, Hegnerova K, Piliarik M et al (2010) Biosens Bioelectron 26:1231–1238
35. Fernandez F, Pinacho DG, Sanchez-Baeza F et al (2011) J Agric Food Chem 59:5036–5043
36. Xiang Y, Lu Y (2011) Nat Chem 3:697–703
37. Grossi M, Lanzoni M, Pompei A et al (2010) Biosens Bioelectron 26:983–990
38. Stevens RC, Soelberg SD, Eberhart BL et al (2007) Harmful Algae 6:166–174
39. Taitt CR, Shriver-Lake LC, Ngundi MM et al (2008) Sensors 8:8361–8377
40. Ligler FS, Sapsford KE, Golden JP et al (2007) Anal Sci 23:5–10
41. General Electric (2011) Biacore Q. Available via http://www. biacore.com/food/index.html?setsession=5c442a06a2ccec7b39e 07adc96beabbe. Accessed 2 May 2011
42. Fodey T, Leonard P, O'Mahony J et al (2011) Trends Anal Chem 30:254–269
43. Hanson Technologies HLAB-2020. http://hanson.arqtech.net/ html/1,1128,Products_HLAB_2020,00.html. Accessed 2 May 2011
44. Foods Standards Agency (2002) Measuring foodborne illness levels. Available via http://www.food.gov.uk/safereating/microbiology/ 58736. Accessed 24 Nov 2010
45. European Food Safety Authority (2010) EFSA J 8:1496–1864
46. Wang L, Zhao W, O'Donoghue MB et al (2007) Bioconjug Chem 18:297–301
47. Pal S, Ying W, Alocija EC et al (2008) Biosyst Eng 99:461–468
48. Banerjee P, Bhunia AK (2010) Biosens Bioelectron 26:99–106
49. Bai S, Zhao J, Zhang Y et al (2010) Appl Microbiol Biotechnol 86:983–990
50. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs
51. Shriver-Lake LC, Erickson JS, Sapsford KE et al (2007) Anal Lett 40:3219–3231
52. Shabani A, Zourob M, Allain B et al (2008) Anal Chem 80:9475–9482
53. Valadez AM, Lana CA, Tu S et al (2009) Sensors 9:5810–5824
54. Heidenreich B, Poehlmann C, Sprinzl M et al (2010) Fleischwirtschaft 90:96–99
55. Lakshmanan RS, Guntupalli R, Hu J et al (2007) Sens Actuators B Chem 126:544–550
56. Lin Y, Chen S, Chuang Y et al (2008) Biosens Bioelectron 23:1832–1837
57. Zordan MD, Grafton MMG, Acharya G et al (2009) Cytometry A 75:155–162
58. Lan Y, Wang S, Yin Y et al (2008) J Bionic Eng 5:239–246
59. Ravindranath SP, Mauer LJ, Deb-Roy C et al (2009) Anal Chem 81:2840–2846
60. Chen S, Wu VCH, Chuang Y et al (2008) J Microbiol Methods 73:7–17
61. Poehlmann C, Wang Y, Humenik M et al (2009) Biosens Bioelectron 24:2766–2771
62. Linman MJ, Sugerman K, Cheng Q (2010) Sens Actuators B Chem 145:613–619
63. Xu S, Mutharasan R (2009) Sens Actuators B Chem 143:144–151
64. Branen JR, Hass MJ, Douthit ER et al (2007) J Food Prot 70:841–850
65. Ohk SH, Koo OK, Sen T et al (2010) J Appl Microbiol 109:808–817
66. Luo J, Liu X, Tian Q et al (2009) Anal Biochem 394:1–6
67. Mata D, Bejarano D, Botero ML et al (2010) Electrochim Acta 55:4261–4266
68. Karsunke XYZ, Niessner R, Seidel M (2009) Anal Bioanal Chem 395:1623–1630
69. Koets M, van der Wijk T, van Eemeren JTWM et al (2009) Biosens Bioelectron 24:1893–1898
70. Feltis BN, Sexton BA, Glenn FL et al (2008) Biosens Bioelectron 23:1131–1136
71. Meneely J, Fodey T, Armstrong L et al (2010) J Agric Food Chem 58:8936–8941
72. Pauly D, Kirchner S, Stoermann B et al (2009) Analyst 134:2028–2039
73. Tsai W, Li I (2009) Sens Actuators B Chem 136:8–12
74. Maraldo D, Mutharasan R (2007) Anal Chem 79:7636–7643
75. Yang M, Sun S, Bruck HA et al (2010) Biosens Bioelectron 25:2573–2578
76. Mak AC, Osterfeld SJ, Yu H et al (2010) Biosens Bioelectron 25:1635–1639
77. Wang X, Wang S (2008) Sensors 8:6045–6054
78. Pohanka M, Malir F, Roubal T et al (2008) Anal Lett 41:2344–2353
79. Paniel N, Radoi A, Marty JL (2010) Sensors 10:9439–9448
80. Valimaa A, Kivisto AT, Leskinen PI et al (2010) J Microbiol Methods 80:44–48
81. Commission Regulation (EC) 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products
82. Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins

83. Meneely JP, Sulyok M, Baumgartner S et al (2010) Talanta 81:630–636
84. Hodnik V, Anderluh G (2009) Sensors 9:1339–1354
85. Wang Y, Dostalek J, Knoll W (2009) Biosens Bioelectron 24:2264–2267
86. Wang Z, Duan N, Hun X et al (2010) Anal Bioanal Chem 398:2125–2132
87. Botana LM, Alfonso A, Botana A et al (2009) Trends Anal Chem 28:603–611
88. Fusetani N, Kem W (2009) Prog Mol Subcell Biol 46:1–44
89. Dominguez HJ, Paz B, Daranas AH et al (2010) Toxicon 56:191–217
90. Campas M, Prieto-Simon B, Marty J (2007) Talanta 72:884–895
91. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin
92. Vilarino N, Carmen Louzao M, Vieytes MR et al (2010) Anal Bioanal Chem 397:1673–1681
93. Fonfria ES, Vilarino N, Campbell K et al (2007) Anal Chem 79:6303–6311
94. Llamas NM, Stewart L, Fodey T et al (2007) Anal Bioanal Chem 389:581–587
95. Stewart LD, Elliott CT, Walker AD et al (2009) Toxicon 54:491–498
96. Stewart LD, Hess P, Connolly L et al (2009) Anal Chem 81:10208–10214
97. Marchesini GR, Hooijerink H, Haasnoot W et al (2009) Trends Anal Chem 28:792–803
98. Health and Safety Executive (2009) What are pesticides? Available via http://www.pesticides.gov.uk/about_pesticides.asp?id=219. Accessed 29 Nov 2010
99. European Food Safety Authority (2010) Pesticides. Available via http://www.efsa.europa.eu/en/prapertopics/topic/pesticides.htm. Accessed 29 Nov 2010
100. Fintschenko Y, Krynitsky AJ, Wong JW (2010) J Agric Food Chem 58:5859–5861
101. US Environmental Protection Agency (2010) Types of pesticides. Available via http://www.epa.gov/pesticides/about/types.htm. Accessed 29 Nov 2010
102. US Environmental Protection Agency (2009) Pesticides and food: what the pesticide residue limits are on food. Available via http://www.epa.gov/pesticides/food/viewtols.htm. Accessed 29 Nov 2010
103. Liu S, Yuan L, Yue X et al (2008) Adv Powder Technol 19:419–441
104. Nagatani N, Takeuchi A, Hossain MA et al (2007) Food Control 18:914–920
105. Caetano J, Machado SAS (2008) Sens Actuators B Chem 129:40–46
106. European Food Safety Authority (2010) EFSA J 8:1646–2088
107. Council Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC
108. Roepcke CBS, Muench SB, Schulze H et al (2010) J Agric Food Chem 58:8748–8756
109. Vidal JC, Bonel L, Castillo JR (2008) Electroanalysis 20:865–873
110. Buonasera K, Pezzotti G, Scognamiglio V et al (2010) J Agric Food Chem 58:5982–5990
111. Sanchez MT, Flores-Rojas K, Guerrero JE et al (2010) Pest Manag Sci 66:580–586
112. Piao YZ, Kim YJ, Kim YA et al (2009) J Agric Food Chem 57:10004–10013
113. Chen Y, Ren HL, Liu N et al (2010) J Agric Food Chem 58:8895–8903
114. Blazkova M, Rauch P, Fukal L (2010) Biosens Bioelectron 25:2122–2128
115. Hossain SMZ, Luckham RE, McFadden MJ et al (2009) Anal Chem 81:9055–9064
116. Akkad R, Schwack W (2010) J Chromatogr B 878:1337–1345
117. Sun X, Wang X (2010) Biosens Bioelectron 25:2611–2614
118. Hildebrandt A, Bragos R, Lacorte S et al (2008) Sens Actuators B Chem 133:195–201
119. Kim N, Park I, Kim D (2007) Biosens Bioelectron 22:1593–1599
120. Lee JH, Han YD, Song SY et al (2010) Biochip J 4:223–229
121. Vamvakaki V, Chaniotakis NA (2007) Biosens Bioelectron 22:2848–2853
122. Kim D, Kim N (2008) Biochip J 2:218–221
123. Norouzi P, Pirali-Hamedani M, Ganjali MR et al (2010) Int J Electrochem Sci 5:1434–1446
124. Shim J, Woo J, Moon S et al (2009) J Membr Sci 330:341–348
125. Murthy MR, Mandappa IM, Latha R et al (2010) Anal Methods 2:1355–1359
126. Solanki PR, Prabhakar N, Pandey MK et al (2008) Biomed Microdevices 10:757–767
127. Kaushik A, Solanki PR, Ansari AA et al (2009) Biochem Eng J 46:132–140
128. Virolainen NE, Pikkemaat MG, Elferink JWA et al (2008) J Agric Food Chem 56:11065–11070
129. Huet AC, Charlier C, Weigel S et al (2009) Food Addit Contam Part A Chem 26:1341–1347
130. Huet AC, Charlier C, Singh G et al (2008) Anal Chim Acta 623:195–203
131. Chen B, Ma M, Su X (2010) Anal Chim Acta 674:89–95
132. Council Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down a Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council
133. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin
134. Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/ EEC and 86/469/EEC and Decisions 89/187/EEC and 91/ 664/EEC
135. US Federal Government 21 CFR 556 Code of Federal Regulations, title 21: food and drugs, part 556-tolerances for residues of new animal drugs in food
136. US Federal Government 9 CFR 310 Code of Federal Regulations, title 9: animals and animal products, part 310-post-mortem inspection
137. US Department of Agriculture, Food Safety and Inspection Service (2011) Residue testing; national residue program. Available via http://www.fsis.usda.gov/Science/Chemistry/index. asp#NRP. Accessed 19 Jan 2011
138. Raz SR, Bremer MGEG, Haasnoot W et al (2009) Anal Chem 81:7743–7749
139. Connolly L, Thompson CS, Haughey SA et al (2007) Anal Chim Acta 598:155–161
140. Thompson CS, Traynor IM, Fodey TL et al (2009) Anal Chim Acta 637:259–264
141. Jornet D, Gonzalez-Martinez MA, Puchades R et al (2010) Talanta 81:1585–1592
142. Spinks CA (2000) Trends Food Sci Technol 11:210–217
143. Haasnoot W, Gercek H, Cazemier G et al (2007) Anal Chim Acta 586:312–318

144. Marchesini GR, Haasnoot W, Delahaut P et al (2007) Anal Chim Acta 586:259–268
145. Adrian J, Pasche S, Pinacho DG et al (2009) Trends Anal Chem 28:769–777
146. Fodey T, Murilla G, Cannavan A et al (2007) Anal Chim Acta 592:51–57
147. Campbell K, McGrath T, Sjölander S et al (2011) Biosens Bioelectron 26:3029–3036
148. Uludag Y, Tothill IE (2010) Talanta 82:277–282
149. Thomas E, Bouma A, van Eerden E et al (2006) J Immunol Methods 315:68–74
150. Davis F, Higson SPJ (2010) Pediatr Res 67:476–480
151. Blake CJ (2007) Anal Bioanal Chem 389:63–76
152. Elenis DS, Kalogianni DP, Glynou K et al (2008) Anal Bioanal Chem 392:347–354
153. Lim M, Ye H, Panoskaltsis N et al (2007) Biotechnol Adv 25:353–368
154. Metfies K, Diercks S, Schroeder F et al (2009) Oceans 2009—Europe, vol 2. pp 1092–1098
155. Kumlanghan A, Kanatharana P, Asawatreratanakul P et al (2008) Enzyme Microb Technol 42:483–491
156. Smith RG, D'Souza N, Nicklin S (2008) Analyst 133:571–584
157. Bronner V, Denkberg G, Peled M et al (2010) Anal Biochem 406:147–156
158. Ben Rejeb I, Arduini F, Arvinte A et al (2009) Biosens Bioelectron 24:1962–1968
159. Asuncion Alonso-Lomillo M, Dominguez-Renedo O, Ferreira-Goncalves L et al (2010) Biosens Bioelectron 25:1333–1337
160. Panini NV, Bertolino FA, Salinas E et al (2010) Biochem Eng J 51:7–13
161. Wang X, Tao G, Meng Y (2009) Anal Sci 25:1409–1413
162. Yuan J, Oliver R, Aguilar M et al (2008) Anal Chem 80:8329–8333
163. Moeller N, Mueller-Seitz E, Scholz O et al (2007) Eur Food Res Technol 224:285–292
164. Campanella L, Di Persio G, Pintore M et al (2009) Food Technol Biotechnol 47:172–177
165. Keegan J, Whelan M, Danaher M et al (2009) Anal Chim Acta 654:111–119