JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
SYMPOSIUM INTRODUCTION

DOI:10.1021/jf904599w

Emerging Pesticide Residue Issues and Analytical Approaches†

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The 46th Annual Florida Pesticide Residue Workshop of 2009 (FPRW 2009) held in St. Pete Beach, FL, is the latest in an annual tradition drawing scientists from U.S. federal and state government laboratories, industry, and other laboratories worldwide. In 2009, selected FPRW presenters were invited to contribute to this special issue of the *Journal of Agricultural and Food Chemistry* with a section devoted to emerging pesticide residue issues and analytical approaches. What follows is the written record of what should become a scientific conversation launched at FPRW 2009. There are two distinct approaches to organic residue analysis: instrumental methods and assays. In much of the world, scientists primarily rely on laboratories equipped with instrumentation for analysis, usually gas chromatography and liquid chromatography with some type of selective detector. In the discussion of instrumental approaches, the focus is on chromatography with mass spectrometry as a detection method. Approaches such as biomonitoring and assays fall outside the traditional instrumental method approach to residue analysis. Assays that do not require laboratory equipment are of greater interest for screening and are well-suited to field use. Regardless of the analytical method, the success of multiresidue analysis relies on the appropriate choice of sample preparation and cleanup methodologies. Many new sample preparation and cleanup approaches used for pesticide and other small molecule contaminant residue analyses in a variety of complex sample matrices are discussed in this special issue. The goal of these approaches is to reduce overall analysis time and solvent consumption without compromising the analytical results.

KEYWORDS: Pesticide residues; organic residue analysis; instrumentation; bioassay; chromatography; mass spectrometry

INTRODUCTION

The primary driver behind residue analysis remains regulatory compliance. To ensure the economic success of a crop or food product in a particular market, the local requirements for maximum residue levels (MRL) for a variety of pesticides must be met. Additionally, the methods used to determine the presence or absence of chemical residues at the legally acceptable limit must meet the standards set by law. Every region and nation has its own approaches to this. In the European Community, the levels and performance criteria of methods are proscribed by EC law. In the United States, many methods are proscribed as well. Because of the wide variety of complex sample matrices, it is the challenge of the analytical chemist to develop analytical approaches to meet or exceed the requirements with regard to the detection of analyte and method sensitivity of whatever legislation they are bound to follow. For this reason, selected papers from the 46th Annual Florida Pesticide Residue Workshop of 2009 (FPRW 2009) held in St. Pete Beach, FL, highlighting the emerging pesticide residue issues and approaches are included in this special issue to publicize the analytical challenges and describe successful approaches. What follows is not an in-depth review of pesticide residue analysis, but rather a brief introduction and background to the most successful approaches and important issues in pesticide residue analysis, chiefly chromatography−mass spectrometry, biomonitoring, and sample preparation, for the purposes of putting the FPRW 2009 scientific dialogue into context.

CHROMATOGRAPHY−MASS SPECTROMETRY

There are approximately 1000 pesticide active ingredients, recognized worldwide, with more than 500 pesticides and metabolites registered and regulated in the United States (*1*−*4*). These include the applied active ingredients and their respective degradation compounds. Methods are sensitive not only to the target analyte(s) but also to the food matrix in which they are found (*5*). Prior to the mid-1990s, the number of samples to be analyzed using a relatively costly chromatography−single-stage mass spectrometry detection method drove the analytical community to explore less costly affinity binding assays for single analytes. However, as the number of residues analyzed per commodity has increased, more and more methods are chromatography−mass spectrometry-based multiresidue methods relying then on the

†Part of the Florida Pesticide Residue Workshop 2009.

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Published on Web 05/04/2010

resolution and speed of the chromatographic method coupled to the identification, confirmation, and low limits of detection of the instrumental method (*2*). Since the mid-1990s, gas chromatography−mass spectrometry (GC-MS), followed by liquid chromatography−mass spectrometry (LC-MS), has become more commercially available and affordable.

Although selective GC detectors such as the nitrogen−phosphorus detector (NPD), electrolytic conductivity detector (ELCD), flame photometric detector (FPD), and single-stage GC-MS are still employed (*6*), the challenges in identification have driven many scientists to use hyphenated mass spectrometry detection methods, usually tandem mass spectrometry (MS/MS), for greater specificity and structural identification (*6*). To maximize instrument sensitivity, this is typically done in a targeted approach. If a single-quadrupole mass spectrometer is used, the detector is often used in a selected ion monitoring (SIM) mode rather than full scan. For example, Podhorniak et al. were able to optimize single-quadrupole LC-MS in the SIM mode for the high-throughput analysis of formetanate HCl in peach and nectarine samples.

In hyphenated mass spectrometry, typically employing triple-quadrupole technology, a selective reaction monitoring (SRM) mode is preferably employed (*6*). In this special issue, Zhang et al. and Wong et al. present compelling examples of the power of GC-MS/MS for multiresidue analysis in difficult or diverse matrices such as ginseng and fresh produce. Wang et al. demonstrate the power of LC-MS/MS and ultrahigh-performance liquid chromatography−quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS) in a method for the determination of 148 pesticides in berries. Kamel takes the tandem mass spectrometric approach for a single insecticide residue and its metabolites describing a methodology for the determination of imidacloprid and its metabolites in bees and bee products by LC-MS/MS.

Martos et al. demonstrate the power of multiclass, multiresidue drug analysis in animal tissue using LC-MS/MS. Likewise, Yang et al. describe an interlaboratory validation study of an LC-MS/MS method for multiresidue pesticide analysis in foods.

SAMPLE PREPARATION CHALLENGES AND APPROACHES

Regardless of analytical approach, sample preparation remains the biggest challenge for multiresidue contaminant analysis in food. With an expanding list of hundreds of residues to measure, and an equally expansive list of tens of thousands of matrices, the challenges for extraction of multiple residues per commodity are legion. However, a relatively straightforward sample preparation method and its variations has taken hold: Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) (*7*). This manual method simplifies and multiplexes extraction and sample cleanup. Several authors presented papers during FPRW 2009 describing how they have modified the QuEChERS method to perform well to meet different challenges such as those posed by difficult matrices such as feed, cereals, seeds, nuts, and dough. In this special issue Kang et al. demonstrate a modified QuEChERS method for a single target, cyromazine, in poultry feed. Lehotay et al. describe modifications to QuEChERS applied to a multiresidue extraction of pesticides in flaxseed, peanut, dough, and cereal grains. The simplicity and wide applicability of QuEChERS and its modifications are evident in the number of presentations discussing this sample preparation with both LC- and GC-MS analysis of pesticides in challenging matrices.

Other innovators, including instrument manufacturers, have also developed automated methods such as in-line solid phase extraction, disposable pipet extraction (DPX), and turbulent flow chromatography (*8*−*10*). In this special issue Brewer et al. describe the DPX approach to a multiresidue pesticide extraction in fruits and vegetables using micro-Luke (*11*). These DPX tips greatly reduce sample preparation time and solvent consumption when compared to conventional solid phase extraction techniques.

BIOMONITORING AND ASSAYS

No scientific discussion of residue monitoring would be complete without a discussion of biomonitoring and assays (*12*). In this special issue, Buonasera et al. report on work that demonstrates the utility of a biosensor as a prescreen to aid in the overall laboratory work flow for pesticide analysis. The biosensing instruments reported here are based on amperometric and optical mechanisms, respectively, to produce an electric current and a luminescence/fluorescence variation when the biomolecules, interfaced with an electrode or a photodiode, react with a pesticide molecule in the sample solution. Atrazine, diuron, linuron, and terbuthylazine have been detected at concentrations as low as 10 nM by the use of photosynthetic protein complexes extracted from algae and higher plants. These protein complexes contain a specific binding site for the herbicide. Molecular biology techniques succeeded in producing an array of biomediator mutants. These biomediator mutants, having different specificities and resistances to different classes and subclasses of pesticides, offer a fast means for prescreening of pesticides and for conducting total toxicology analysis. By using biosensors, only samples giving positive results at the required sensitivity for enforcement would require more detailed and reliable instrumental analyses such as HPLC and GC-MS.

CONCLUSIONS

The primary purpose of this literature conversation is a description of tools and innovation applied in ways that are fit to purpose. There is no single approach to residue analysis in food that fits all needs. Modifications due to matrix, target, and laboratory resources must be made. Each contributor to this special issue has found a solution that fits the boundaries of the sample and the laboratory and described their results given those boundary conditions. The problems and challenges of residue analysis may be legion, but, fortunately, the tools and creativity of those applying them are also legion.

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Received for review December 30, 2009.