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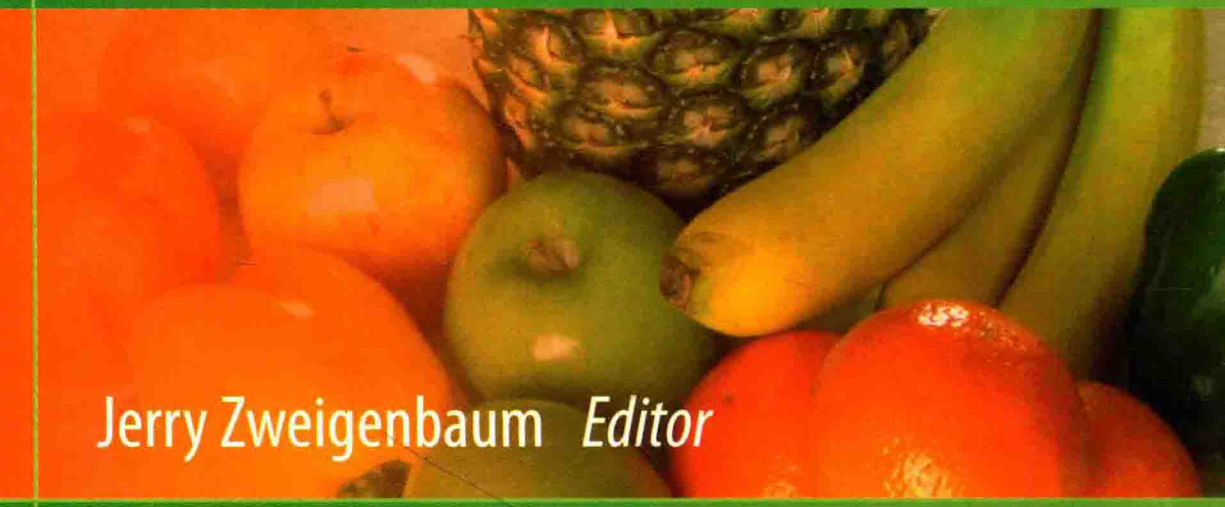

Jerry Zweigenbaum *Editor*

Mass Spectrometry in Food Safety

Methods and Protocols

Humana Press

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Edited by

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Preface

Food is a complex biological material for which all life on the planet depends and is intertwined with all living things. Thus, the food chain is both a synergistic and competitive system between plants and animals. No one need be reminded that it is a key component of human survival and that we are a part of that system, albeit on the top of that chain. A safe and sufficient food supply is necessary for a healthy and productive population throughout the world. In today's world, food for human consumption is not a local commodity but is obtained through a network of supply and transportation that spans all points of the globe. Nuts from Turkey, fruits from Chili, and shrimp from Vietnam can appear at a local grocery store anywhere in the world.

Bacterial infestation is a major cause of acute toxicity from food and has brought public awareness to pathogenic testing. Where *Salmonella*, *Listeria*, *E. coli*, and other food-borne pathogens have caused sudden and serious (even sometimes fatal) outbreaks, public attention becomes highly focused on the need to assure a safe food supply. As insidious, or maybe even more so, is the possible continued exposure to chemical residues of pesticides, veterinary drugs, chemical contaminants, and naturally produced chemical toxins, such as mycotoxins. This chemical threat to the food supply usually represents chronic toxicity and does not gain the attention that acutely toxic events command. However, as in the case of melamine adulteration, where public awareness was heightened by the acute toxicity incurred, the possibility of chemical contamination of our food remains a serious threat that demands continuous attention.

Because of the competition for fruits, grains, and vegetables with insects, rodents, other small animals, and birds, the use of pesticides is a necessary supplement for farmers to obtain good yields to feed a growing population of people around the world. Through risk assessment and proper application, the use of pesticides is a safe way to assure sufficient food for the world's people. However, the possibility of exposure to elevated levels or to pesticides no longer approved for use places people at risk of chronic toxicity with implications impacting human health from cancer to possible behavior modification. For example, recent studies have implicated the possibility of a correlation with autism and attention deficit disorder. Because of the long-term effects and slow manifestation of chronic exposure, this threat to our food supply may indeed be more insidious than an acute toxic exposure.

Likewise, veterinary drugs are necessary to assure healthy animals and their products that are used for food (e.g., milk, eggs, etc.). However, there are antibiotics that have been banned because of their toxicity to humans. In addition, the overuse of approved antibiotics may cause drug-resistant bacterial strains, and exposure of veterinary drugs to humans through the food supply may directly impact human health. The use of hormones to increase yields for animal production may have deleterious effect and are banned in some parts of the world. This places even yet another dilemma for food producers; where hormones are allowed, meat and animal products may contain residual amounts, and these foods should only be imported to regions where they are not banned. With a world food supply, this is difficult and more disconcerting, in terms of a safe food supply, and it would appear that harmonized good science and practice would be in the best interest of the entire world's population.

A third area of chemical concern to the world's food supply is that of naturally produced toxins. Among these is the category of mycotoxins or toxins that are produced as secondary metabolites of fungi. Unlike bacteria that has to be a live viable organism to cause deleterious health effects, mycotoxins, once produced, are refractory small molecules that have resident times long after the fungus that produced them are gone. Among these are the aflatoxins that are known carcinogens. There are many other mycotoxins that are found in fruits, vegetables, spices, and grains and affect not only people that eat them directly but wildlife and livestock. Again these toxins represent a threat to the food supply, where the insidious effects of long-term chronic toxicity make it difficult to chart their impact on human health. However, scientists around the world are aware of their effects if not actually able to quantify them except in regions of extreme exposure.

The final area of chemical threat to our food supply is that of contaminants. This broad range of chemicals is found in the environment, in processing, and in the packaging of food. This category of residues is classified as those materials that are neither intentionally nor naturally found in our food. One cause of this chemical contamination is the migration of unwanted bi-products of packaging materials into the food. Packaging material is an important component of the safe shipping and preservation of foodstuff and is continually tested to assure that unwanted chemicals are not found in and do not migrate from the packaging material into the food. Packaging material includes plastics bags, coatings of cans, and any other containment of food and beverages. The other route of contaminants through the environment often occurs in the form of persistent organic pollutants or POPS. These compounds remain in the environment long after their use has been banished from society. An example is that of polychlorinated biphenyls which were used exclusively through the 1970s as insulators in transformers and capacitors until their ban in the end of that decade. These compounds are still found in air (dust), water, and soil and do make their way into the food supply.

It is my opinion that total elimination of all the above in the world's food supply is simply impossible. However, keeping harmful chemical residues within acceptable risk levels is not only scientifically reasonable, but also a responsibility that all societies owe each other. The only way to accomplish this is through regulation, and it is for this reason that this book begins with an overview of the regulations around the world. Few dispute that the European Union has led the world in the most up-to-date regulations following sound scientific studies of risk assessment leading to reasonable regulations to meet the goal of ensuring a safe food supply. To give a global perspective, a view of the food safety regulations of China, the USA, and Japan are also given. These four regulatory bodies have both a great influence and stake in both import and export of food throughout the world.

The only way to monitor and enforce these regulations is through extensive food testing, and that is the subject of the remainder of this book. Mass spectrometry has become the enabling technology for both identifying and quantifying low-level chemical residues in one of the most complex biological matrices: food. Even with its high degree of chemical selectivity, or its capability to distinguish one chemical from another, the need for good sample preparation remains. Thus, the next two chapters cover two powerful procedures that have become companions to the powerful techniques of tandem mass spectrometry. The preparative technique known as QuEChERS has become a routine procedure in laboratories performing complex multiresidue pesticide analysis and has found its way into many other applications, including most recently the determination of contaminants in the Gulf of Mexico's oil spill. In addition to this manual approach, automated sample preparation offers its advantages, and thus the reader is offered the opportunity to compare and contrast these important aspects of sample preparation.

The next three chapters cover the complex aspects of testing food samples for pesticide residues. Each chapter covers chromatographic techniques combined with mass spectrometry. Gas chromatography/mass spectrometry has been used for many years for pesticide residue analysis, but even these techniques have experienced rapid advances in recent years, which are covered. The approval and use of more polar pesticides combined with shipment of fresh produce around the world has contributed to the need for rapid analysis, and liquid chromatography/tandem mass spectrometry has advanced to meet that need. The complex procedures and considerations are covered using that technology. Finally, the identification of unexpected or nontargeted pesticides has become increasingly of concern, and mass spectrometry advances that address this need conclude the contributions in this book for pesticide analysis.

Mycotoxins continue to be of major concern to scientists and regulators throughout the world. Most monitoring has centered on the aflatoxins, and there are relatively selective methods for their determination in common use, mainly liquid chromatography combined with fluorescent detection. However, other mycotoxins that do not respond to this technology are finding mass spectrometry to be the analytical method of choice. Methods for some of these residues are given. In the area of testing of antibiotics, an excellent overview is given. This is followed by detailed methodology for monitoring specific antibiotics in both animal and animal products. Likewise, the need to determine hormones and the methods used are described. These chapters combined give the reader an excellent perspective of the requirements for testing veterinary drugs and how mass spectrometry meets the needs of the present day analytical food laboratory.

The final chapters of this book cover the area of chemical contaminants. The description of present day methods for evaluating packaging materials provides in-depth insight. The complex analysis of persistent organic pollutants is thoroughly reviewed. The reader will find that both the overviews and the specific methods provide a comprehensive picture of the state of chemical residue food monitoring in the 21st century. In addition, the contributors represent scientists engaged in food safety from around the world, and thus it is a world perspective. It is this editor's hope that each reader will gain both understanding and appreciation for the contribution of mass spectrometry and those who pioneer its use as it is applied to food testing and food safety.

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Contents

Chapter 1

European Union Regulations

Peter Fürst

Abstract

The European Union (EU) has been a leader in the development of both guidance and regulations to ensure food safety throughout the member states. Because of the free movement of food commodities among the countries that belong to the European Union, there is a great need to assure high quality monitoring of both imported food and member state products. The procedures and methods required need to be practical, state-of-the art, and harmonised. The European Commission has developed a network of laboratories and scientific studies to meet this goal. This chapter describes the current Regulations, Directives and Decisions of the European Commission that protect the food supply throughout Europe. Because imported food needs to comply with the EU requirements, and the need to have common compliance throughout the member states, the developed system could be a worldwide template for monitoring the food supply. In addition, the integral role of chromatography hyphenated to mass spectrometry is described.

Key words: European Union, Regulations, Guidance, Directives, Decisions

1. Introduction

The European Union (EU) is an economic and political union of currently 27 Member States with a total of almost 500 million citizens. Since its foundation it has developed a single market through a standardised system of laws which apply in all Member States. The single market guarantees a free movement of people, goods, services, and capital. Treaties (known as "primary" legislation) are the basis for a large body of "secondary" legislation which has a direct impact on the daily lives of EU citizens. Secondary legislation consists mainly of Regulations, Directives, Decisions, and Recommendations adopted by the EU institutions. While Regulations have direct effect and are binding in all Member States, Directives require implementation by national

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legislation to be effective. In contrast, Decisions only affect those parties to whom they are addressed. The laws, along with EU policies in general, are the result of decisions taken by the institutional triangle made up of the Council which represent national governments, the European Parliament and the European Commission which is responsible for initiating legislation.

Direct free access to European Union Law is provided by EUR-Lex (http://eur-lex.europa.eu/en/index.htm) which contains the Official Journal (OJ) as well as the treaties, legislation, legislative proposals, and in addition offers extensive search facilities.

The general principles and requirements governing food and feed in general, and food and feed safety in particular, at the Community and national level are laid down in Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 (1). As laid down in Article 1, "this Regulation provides the basis for the assurance of a high level of protection of human health and consumers' interest in relation to food, taking into account in particular the diversity in the supply of food including traditional products, whilst ensuring the effective functioning of the internal market. It establishes common principles and responsibilities, the means to provide a strong science base, efficient organisational arrangements, and procedures to underpin decision making in matters of food and feed safety."

Through this Regulation, the European Food Safety Authority (EFSA) and the Rapid Alert System for Food and Feed (RASFF) are established. According to Article 22, EFSA "shall provide scientific advice and scientific and technical support for the Community's legislation and policies in all fields which have a direct or indirect impact on food and feed safety. It shall provide independent information on all matters within these fields and communicate on risks." Thus, EFSA is responsible for risk assessment, whereas the European Commission is in charge of risk management measures. Whereas, the purpose of the RASFF is to provide the control authorities with an effective tool for exchange of information on measures taken to ensure food safety by establishing a network for the notification of a direct or indirect risk to human health deriving from food or feed.

While the basic rules with regard to the food and feed law are laid down in Regulation (EC) No. 178/2002, a specific harmonised framework of general rules for the organization of official controls at the Community level are established by Regulation (EC) No. 882/2004 of the European Parliament and of the Council of 29 April 2004 (2). The general requirements for methods of sampling and analysis and laboratories are laid down in Articles 11 and 12.

Article 11 stipulates that sampling and analysis methods used in the context of official controls shall comply with relevant Community rules; or (a) if no such rules exist, with internationally

recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation; or(b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols.

Where the above does not apply, validation of methods of analysis may take place within a single laboratory according to an internationally accepted protocol. Wherever possible, methods of analysis shall be characterised by the following appropriate criteria:

- Accuracy
- Applicability (matrix and concentration range)
- Limit of detection
- Limit of determination
- Precision
- Repeatability
- Reproducibility
- Recovery
- Selectivity
- Sensitivity
- Linearity
- Measurement uncertainty
- Other criteria that may be selected as required

Article 11 also establishes that the following implementing measures may be taken by the Commission:

- Methods of sampling and analysis, including the confirmatory or reference methods to be used in the event of a dispute.
- Performance criteria, analysis parameters, measurement uncertainty, and procedures for the validation of the before mentioned methods.
- Rules on the interpretation of results.

In any case, samples must be handled and labelled in such a way as to guarantee both their legal and analytical validity.

According to Article 12 of this Regulation, the competent authority of the Member States shall designate laboratories that may carry out the analysis of samples taken during official controls. However, they may only designate laboratories that operate and are assessed and accredited in accordance with the following European standards:

- EN ISO/IEC 17025 on "General requirements for the competence of testing and calibration laboratories."

- EN ISO/IEC 17011 on "General requirements for accreditation bodies accrediting conformity assessment bodies."
- Taking into account criteria for different testing methods laid down in Community feed and food law.

The accreditation and assessment of testing laboratories may relate to individual tests or groups of tests.

In order to contribute to a high quality and uniformity of analytical results, an analytical network of European Reference Laboratories (EURL), formerly called "Community Reference Laboratories (CRL)", National Reference Laboratories (NRL), and Official National Laboratories (OFL) was designated in the past for various classes of analytes. The activities of reference laboratories cover all areas of feed and food law and animal health, in particular those areas where there is a need for precise analytical and diagnostic results.

Article 32 of Regulation (EC) No. 882/2004 lays down the following major responsibilities for EURL for food and feed:

- Providing NRL with details of analytical methods, including reference methods.
- Coordinating application by the NRL of those methods, in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols, when available.
- Coordinating, within their area of competence, practical arrangements needed to apply new analytical methods and informing NRL of advances in this field.
- Conducting initial and further training courses for the benefit of staff from NRL and of experts from developing countries.
- Providing scientific and technical assistance to the Commission, especially in cases where Member States contest the results of analyses.
- Collaborating with laboratories responsible for analysing feed and food in third countries.

According to Article 33 of Regulation (EC) No. 882/2004 Member States shall arrange for the designation of one or more NRL for each EURL. These NRL inter alia shall

- Collaborate with the EURL in their area of competence.
- Coordinate, for their area of competence, the activities of OFL responsible for the analysis of samples.
- Where appropriate, organise comparative tests between the OFL and ensure an appropriate follow-up of such comparative testing.
- Ensure the dissemination to the Competent Authority and OFL of information that the EURL supplies.

- Provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans.

While the Regulations (EC) No. 178/2002 and 882/2004 contain the general principles and requirements, specific analytical requirements as well as maximum levels for a number of residues and contaminants are laid down in special legislation. Regarding analytical methods, the EU generally follows the criteria approach. This means that no fixed methods are prescribed but detailed and strict performance criteria are established by the Commission which have to be fulfilled. As long as it can be demonstrated in a traceable manor that these performance criteria are fulfilled and the method is fit for purpose the analysts can apply whatever method. The great advantage of this approach is that it does not impede the fast innovation and progress in analytical instrumentation.

2. Pesticides

Until 1 September 2008, the legislation for pesticide residues was a shared responsibility of the European Commission and the Member States. Since 1976, more than 45,000 Community maximum residue levels (MRLs) were set for various commodities for 245 pesticides on cereals (Directive 86/362/EEC), foodstuffs of animal origin (Directive 86/363/EEC), fruit and vegetables, and other plant products (Directive 76/895/EEC and Directive 90/642/EEC). For the tens of thousands of pesticide/commodity combinations for which no Community MRLs existed, Member States could set MRLs at national level to facilitate trade and to protect the health of their consumers.

As from 1 September 2008, Regulation (EC) No. 396/2005 of the European Parliament and of the Council on MRLs of pesticides in or on food and feed of plant and animal origin defines a new fully harmonised set of rules for pesticide residues (3). This Regulation simplifies the existing legislation by harmonising pesticide MRLs and making them directly applicable in all Member States. The annexes to Regulation (EC) No. 396/2005 specify the MRLs and the products to which they apply.

Annex I is the list of products to which the MRLs apply. Annex I has been established by Commission Regulation (EC) No. 178/2006. It contains 315 products, including fruits, vegetables, spices, cereals, and animal products.

Annex II is the list of EU definitive MRLs and it consolidates the existing EU legislation before 1 September 2008. It specifies MRLs for 245 pesticides.

Annex III is the list of the so-called EU temporary MRLs. It is the result of the harmonisation process as it lists pesticides for

which, before 1 September 2008, MRLs were only set at national level. It specifies MRLs for 471 pesticides.

Annex IV is the list of currently 52 pesticides for which no MRLs are needed because of their low risk.

Annex V will contain the list of pesticides for which a default limit other than 0.01 mg/kg will apply. This annex has not been published yet.

Annex VI will contain the list of conversion factors of MRLs for processed commodities. This annex has not been published yet.

Annex VII contains a list of pesticides used as fumigants for which the Member States are allowed to apply special derogations before the products are placed on the market (4).

If a pesticide is not included in any of the above mentioned Annexes a default MRL of 0.01 mg/kg applies.

The new pesticide Regulation is the result of a comprehensive review programme that was launched in 1993 by the European Commission for all active substances used in plant protection products within the European Union. In this review process, each substance had to be evaluated as to whether it could be used safely with respect to human health (consumers, farmers, local residents, and passers-by) and the environment, in particular groundwater and non-target organisms, such as birds, mammals, earthworms, and bees. The review of existing pesticides has led to the removal of pesticides from the market which cannot be used safely. Of some 1,000 active substances on the market, in at least one Member State before 1993, about 250 active substances have passed the harmonised EU safety assessment. Almost 700 active substances have been eliminated because dossiers were not submitted, incomplete, or withdrawn by industry. About 70 substances failed the review and have been removed from the market, because the evaluation carried out did not show safe use with respect to human health and the environment (5).

An EU pesticides database has been created and published on the web that provides a search tool to find out which active substances are approved in Europe together with a reference to the EU legislation. Moreover, this database includes the respective relevant toxicological information and the MRLs in food and feed (6).

The method validation and quality control procedures for pesticide residues analysis in food and feed are laid down in guidance documents published by the Directorate General (DG) for Health and Consumers of the European Commission. This DG has inter alia, the task of keeping the laws on the safety of food and feed up to date. The guidance documents are reviewed and updated regularly. The currently effective requirements (implemented by 01/01/2010) are laid down in the document "SANCO/10684/2009" (7). SANCO is the abbreviation of the French term "Santé et Consommateurs" for "Health and Consumers."

The guidance in this document is intended for the monitoring of pesticide residues in the European Union. The document describes the method of validation and analytical quality control (AQC) requirements to support the validity of data used for checking compliance with maximum residue levels (MRLs), enforcement actions, or assessment of consumer exposure to pesticides.

The key objectives are to

- Provide a harmonised cost-effective quality assurance system in the EU.
- Ensure the quality and comparability of analytical results.
- Ensure that acceptable accuracy is achieved.
- Ensure that false positives or false negatives are not reported.
- Support compliance with ISO/IEC 17025.

The document is complementary and integral to the requirements in ISO/IEC 17025.

Besides detailed requirements, such as for sampling, transport, processing and storage of samples, handling of calibration standards, avoidance of contamination and interferences, performance criteria, confirmation and reporting of results, a number of detailed requirements and recommendations are also laid down concerning mass spectrometry (MS).

The following is an excerpt of SANCO/10684/2009 with respect to application of mass spectrometric techniques in official pesticide analysis:

In case of MRL exceedances or the identification of unusual residues, the use of highly specific detection systems, such as mass spectrometry is recommended.

For GC-MS procedures, the chromatographic separation should be carried out using capillary columns. For LC-MS procedures, the chromatographic separation can be performed using any suitable LC column. In either case, the minimum acceptable retention time for the analyte(s) under examination should be at least twice the retention time corresponding to the void volume of the column. The retention time (or relative retention time) of the analyte in the sample extract must match that of the calibration standard (may need to be matrix matched) within a specified window after taking into consideration the resolving power of the chromatographic system. The ratio of the chromatographic retention time of the analyte to that of a suitable internal standard, i.e. the relative retention time of the analyte, should correspond to that of the calibration solution with a tolerance of ±0.5% for GC and ±2.5% for LC.

Reference spectra for the analyte should be generated using the instruments and techniques employed for analysis of the samples.