

# METHODS IN MOLECULAR BIOLOGY™

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# **Mass Spectrometry in Food Safety**

## **Methods and Protocols**

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.

*Wilmington, DE, USA*

*Jerry Zweigenbaum*





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# Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>xi</i>
1 European Union Regulations . . . . . <i>Peter Fürst</i>	1
2 China's Food Safety Regulation and Mass Spectrometry . . . . . <i>Xiaogang Chu, Feng Zhang, Xuemei Nie, Wenzhi Wang, and Feng Feng</i>	21
3 United States and Japanese Food Regulations . . . . . <i>Jerry Zweigenbaum</i>	53
4 QuEChERS Sample Preparation Approach for Mass Spectrometric Analysis of Pesticide Residues in Foods . . . . . <i>Steven J. Lehotay</i>	65
5 Automated Solid Phase Extraction . . . . . <i>Norbert Helle, Meike Baden, and Kaj Petersen</i>	93
6 Multiresidue Pesticide Analysis by Capillary Gas Chromatography-Mass Spectrometry . . . . . <i>Jon W. Wong, Kai Zhang, Douglas G. Hayward, and Chin Kai-Meng</i>	131
7 Targeted Pesticide Residue Analysis Using Triple Quad LC-MS/MS. . . . . <i>Lutz Alder</i>	173
8 LC/TOF-MS Analysis of Pesticides in Fruits and Vegetables: The Emerging Role of Accurate Mass in the Unambiguous Identification of Pesticides in Food. . . . . <i>Imma Ferrer, E. Michael Thurman, and Jerry Zweigenbaum</i>	193
9 Hormone Analysis in Food Products. . . . . <i>Marco H. Blokland and Saskia S. Sterk</i>	219
10 Analysis of Multiple Mycotoxins in Food . . . . . <i>Jana Hajšlova, Milena Zachariásova, and Tomas Cajka</i>	233
11 Multi Mycotoxin Analysis in Food Products Using Immunoaffinity Extraction . . . . . <i>Masabiko Takino, Hiroki Tanaka, and Toshitsugu Tanaka</i>	259
12 Multiresidue Analysis of Antibiotics in Food of Animal Origin Using Liquid Chromatography–Mass Spectrometry . . . . . <i>Katerina Mastovska</i>	267
13 The LC-MS/MS Methods for the Determination of Specific Antibiotics Residues in Food Matrices . . . . . <i>Gui-Liang Chen and Yan-Yan Fang</i>	309
14 Identification of Unknown Migrants from Food Contact Materials . . . . . <i>Malcolm Driffield, Emma L. Bradley, Laurence Castle, and Leon Coulier</i>	357
15 Halogenated Persistent Organic Pollutants and Polycyclic Aromatic Hydrocarbons in Food . . . . . <i>Tomas Cajka and Jana Hajšlova</i>	373
Erratum . . . . .	E1
<i>Index</i> . . . . .	411



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