eISBN: 9789811421587
ISBN: 9789811421570

CURRENT AND FUTURE DEVELOPMENTS IN FOOD SCIENCE

VOLUME 1

# ADVANCES IN THE DETERMINATION OF XENOBIOTICS IN FOODS

Editors:
**Belén Gómara**
**María Luisa Marina**

Bentham Books

# Current and Future Developments in Food Science

**(*Volume 1*)**

## *Advances in the Determination of Xenobiotics in Foods*

**Edited by**

**Belén Gómara**

*Department of Instrumental Analysis and Environmental Chemistry,*
*Institute of General Organic Chemistry (IQOG),*
*Spanish National Research Council (CSIC),*
*Madrid, Spain*

**&**

**María Luisa Marina**

*Department of Analytical Chemistry,*
*Physical Chemistry and Chemical Engineering,*
*University of Alcalá Alcalá de Henares (Madrid), Madrid, Spain*

**Current and Future Developments in Food Science**

*Volume # 1*

*Advances in the Determination of Xenobiotics in Foods*

Editors: Belén Gómara & María Luisa Marina

ISSN (Online): 2705-0815

ISSN (Print): 2705-0807

ISBN (Online): 9789811421587

ISBN (Print): 9789811421570

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

**Bentham Science Publishers Pte. Ltd.**
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

# CONTENTS

# PREFACE

Xenobiotics had been and presently are of great concern, both for the society and the health authorities all over the world. Xenobiotics in food may include a huge variety of compounds of different nature. Nowadays, one of the groups that have caught the attention of researchers and authorities are food chain residues. These compounds are chemicals unintentionally present in the food due to the different procedures of production and preparation methods to which foodstuffs are subjected. Among them, compounds related to food contact materials such as plasticizers and plastic monomers are one of the xenobiotics mostly supervised by the European Food Safety Authority (EFSA) and the Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) in the United States. In addition, pesticides can also be present in the final foodstuff because of their previous use in the field or on the farm. On the other hand, due to the global distribution of environmental pollutants, they are also susceptible to end up in the food chain because of different processes of deposition and/or bioaccumulation. There are several classes of environmental pollutants. Some of them are regulated by local or global legislations such as persistent organic pollutants and heavy metals. There are also many other emerging contaminants that must be controlled such as some halogenated flame retardants and perfluorinated compounds, among others. In addition, some xenobiotics could be present in the final food consumed as a result of food treatments, as is the case of acrylamide and furan which are related to high-temperature cooking processes. Finally, the presence of natural contaminants such as mycotoxins, aflatoxins and biogenic amines in the final foodstuffs must be controlled too.

The control of all these compounds would not be possible without the development of advanced analytical methodologies enabling their unequivocal, precise and accurate determination in foodstuffs. In this regards, one of the most employed methodologies is the separation techniques coupled to mass spectrometry. Depending on the physicochemical properties of each xenobiotic, gas (GC) or liquid (LC) chromatography can be applied for its separation, identification and quantification. Constant research is being carried out in order to develop more sensitive and selective methods for the determination of these xenobiotics at the low concentration levels they use to be present in foodstuffs. Novel analytical approaches in this field are fast GC and ultra-high performance LC (UHPLC) which have been successfully applied to study some of these xenobiotics. In addition, highly sensitive and selective mass analyzers such as triple quadrupole, Orbitrap or other hybrid systems combining some of them and novel developments such as ion mobility equipment are being recently applied to these purposes. These advances in combination with fast and environmentally friendly sample extraction and purification methods provide the society and authorities with the necessary methods for controlling and regulating, if necessary, the presence of all these xenobiotics in food.

Therefore, this book is aimed to present some of the most recent advances and developments achieved in the determination of different xenobiotics in foods. Chapters are organized according to the type of xenobiotic under study.

This book was inspired by the context of the AVANSECAL-CM and AVANSECAL-II-CM research projects funded by the Comunidad of Madrid and European FEDER program and headed by Professor María Luisa Marina from 2014 to nowadays, which was the continuation of two previous research projects (ANALISYC and ANALISYC-II) headed by Professor María José González from 2006 to 2013. Along all these years, a numerous group of researchers made considerable efforts to develop innovative analytical methodologies to control and improve food quality and safety with very relevant results in this field which have

been and are being recognized at the international level. The editors are very grateful to these researchers, especially to those who have contributed to this e-book, and dedicated this e-book to Professor María José González in her retirement as a worm acknowledgement for her valuable contribution in the field of xenobiotics analysis.

Experts and researchers in analytical chemistry, food safety and xenobiotic analysis and newcomers in these fields such as Ph.D. students or chemists working in control laboratories or laboratory technicians will find in this e-book updated information including a set of advanced analytical methods used for the analysis of a broad spectrum of xenobiotics revealing the most interesting features and drawbacks to be overcome in this field. PhD students will learn more about novel analytical developments, they will acquire knowledge about xenobiotics and know in depth the field of food contamination. Finally, chemists working in control laboratories or laboratory technicians will have a very useful tool to face the problems arising on food safety.

We are very grateful to all the authors for their relevant contributions to this e-book.

Efforts in this field will be pursued in the next four years, thanks to the fundings from the Comunidad of Madrid (Spain) and European FEDER program through the new AVANSECAL-II-CM research project.

**Belén Gómara**
Department of Instrumental Analysis and Environmental Chemistry
Institute of General Organic Chemistry (IQOG)
Spanish National Research Council (CSIC)
Madrid
Spain

**María Luisa Marina**
Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering
University of Alcalá Alcalá de Henares (Madrid)
Madrid
Spain

# DEDICATION

*Dedicated to Professor Maria José González Carlos in her retirement*

# List of Contributors


| | |
|---|---|
| **Alberto Ritieni** | Università di Napoli Federico II, Department of Pharmacy, Via D. Montesano, 49 -, 80131 Napoli, Italy |
| **Beatriz Gómez-Gómez** | Departamento de Química Analítica. Facultad de Ciencias Químicas. Universidad Complutense de Madrid. 28040. Madrid, Spain |
| **Cristina Nerín** | I3A – Aragón Institute of Engineering Research, University of Zaragoza, Calle María de Luna 3, 50018 Zaragoza, Spain |
| **Esther Garrido-Gamarro** | Food Safety and Quality Officer, Fisheries and Aquaculture, Department, Food and Agriculture Organization of the United Nations (FAO). Viale delle Terme di Caracalla, 00153 Roma RM, Italy |
| **Esteban Abad** | Laboratorio de Dioxinas. Departamento de Química Ambiental. IDAEA-CSIC. C/ Jordi Girona, 18-26, 08034 Barcelona, Spain |
| **Ethel Eljarrat** | Institute of Environmental Assessment and Water Research, Spanish National Research Council (IDAEA-CSIC). C/ Jordi Girona, 18-26, 08034 Barcelona, Spain |
| **Filomena Silva** | ARAID – Agencia Aragonesa para la Investigación y el Desarollo, Av. de Ranillas 1-D, planta 2ª, oficina B, 50018 Zaragoza, Spain<br>Faculty of Veterinary Medicine, University of Zaragoza, Calle de Miguel Servet 177, 50013 Zaragoza, Spain |
| **Francisca Holgado** | Institute of Food Science, Technology and Nutrition, Spanish National Research Council (ICTAN-CSIC), C/ José Antonio Novais, 10, 28040 Madrid, Spain |
| **Francisco J. Morales** | Institute of Food Science, Technology and Nutrition, Spanish National Research Council (ICTAN-CSIC), C/ José Antonio Novais, 10, 28040 Madrid, Spain |
| **Gianni Sagratini** | University of Camerino, School of Pharmacy, Via S. Agostino 1, 62032 Camerino (MC), Italy |
| **Giovanni Caprioli** | University of Camerino, School of Pharmacy, Via S. Agostino 1, 62032 Camerino (MC), Italy |
| **Jordi Parera** | Laboratorio de Dioxinas. Departamento de Química Ambiental. IDAEA-CSIC. C/ Jordi Girona, 18-26, 08034 Barcelona, Spain |
| **Kurunthachalam Kannan** | Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, University at Albany, State University of New York, Albany, NY 12201, USA |
| **Manuela Ábalos** | Laboratorio de Dioxinas. Departamento de Química Ambiental. IDAEA-CSIC. C/ Jordi Girona, 18-26, 08034 Barcelona, Spain |
| **Marta Mesías** | Institute of Food Science, Technology and Nutrition, Spanish National Research Council (ICTAN-CSIC), C/ José Antonio Novais, 10, 28040 Madrid, Spain |
| **Massimo Ricciutelli** | University of Camerino, School of Pharmacy, Via S. Agostino 1, 62032 Camerino (MC), Italy |

| | |
|---|---|
| **Òscar Aznar-Alemany** | Institute of Environmental Assessment and Water Research, Spanish National Research Council (IDAEA-CSIC). C/ Jordi Girona, 18-26, 08034 Barcelona, Spain |
| **Pilar Fernández-Hernando** | Departamento de Ciencias Analíticas, Facultad de Ciencias, Universidad Nacional de Educación a Distancia (UNED). Paseo Senda del Rey nº 9, 28040, Madrid, Spain |
| **Qian Wu** | Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, University at Albany, State University of New York, Albany, NY 12201, USA |
| **Raquel Becerril** | I3A – Aragón Institute of Engineering Research, University of Zaragoza, Calle María de Luna 3, 50018 Zaragoza, Spain |
| **Rosa Mª Garcinuño-Martínez** | Departamento de Ciencias Analíticas, Facultad de Ciencias, Universidad Nacional de Educación a Distancia (UNED). Paseo Senda del Rey nº 9, 28040, Madrid, Spain |
| **Sauro Vittori** | University of Camerino, School of Pharmacy, Via S. Agostino 1, 62032 Camerino (MC), Italy |
| **Vicente Andreu** | Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV), Desertification Research Center (CIDE), Joint Research Center CSIC-UV-GV, Moncada, Spain |
| **Yolanda Picó** | Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV), Desertification Research Center (CIDE), Joint Research Center CSIC-UV-GV, Moncada, Spain |
| **Yelko Rodríguez-Carrasco** | University of Valencia, Department of Food Chemistry and Toxicology, Av/ Vicent A. Estellés, s/n 46100 Burjassot,, Valencia, Spain |
| **Yolanda Madrid** | Departamento de Química Analítica. Facultad de Ciencias Químicas. Universidad Complutense de Madrid. 28040. Madrid, Spain |
| **Zoyne Pedrero Zayas** | CNRS/UNIV PAU & PAYS ADOUR, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux, UMR 5254, 64000, Pau, France |

**CHAPTER 1**

# Safety Assessment of Active Food Packaging: Role of Known and Unknown Substances

**Filomena Silva[1,2], Raquel Becerril[3] and Cristina Nerín[3,*]**

[1] *ARAID – Agencia Aragonesa para la Investigación y el Desarollo, Av. de Ranillas 1-D, planta 2ª, oficina B, 50018 Zaragoza, Spain*

[2] *Faculty of Veterinary Medicine, University of Zaragoza, Calle de Miguel Servet 177, 50013 Zaragoza, Spain*

[3] *I3A – Aragón Institute of Engineering Research, University of Zaragoza, Calle María de Luna 3, 50018 Zaragoza, Spain*

**Abstract:** Nowadays, consumers are more aware of what they eat and also request, minimally processed foods and they tend to prefer biodegradable or bio-based packaging. One of the most accepted technologies to battle this problematic is active packaging. Active packaging protects the food product by extending its shelf-life while guaranteeing its safety through the addition of antimicrobials or antioxidants that actively interact with the packaging atmosphere or the food product to avoid oxidation processes, microbial growth and other routes responsible for food spoilage. Although yet not fully implemented in Europe, active packaging is expected to reach a compound annual growth rate of 6.9% in 2020. However, in order to get these active packaging solutions into the market, their safety must be ensured and they must comply with the European legislation on the topic, both for the active substances incorporated into the packaging materials as for the packaging material itself. These packaging materials, either plastic or bio-based, can pose food safety risks to consumers due to the migration of compounds from the packaging to the food product. Compounds like plasticizers, additives, polymer monomers/oligomers and even non-intentionally added substances (NIAS) can migrate from the packaging material to the food product at concentrations capable to endanger human health and, therefore, they must be correctly detected and identified, to allow a correct risk assessment and strict monitoring of the packaging materials available.

**Keywords:** Active packaging, Antioxidant, Antimicrobial, Migration, Release, Food contact materials, Bio-based polymers, Natural compounds, Non-intentionally added substances.

[*] **Corresponding author Cristina Nerín:** Aragón Institute of Engineering Research, Campus Rio Ebro, Edificio Torres Quevedo, Universidad de Zaragoza, Calle María de Luna 3, 50018 Zaragoza, Spain; Tel: +34976761873; E-mail: cnerin@unizar.es

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

## INTRODUCTION

Nowadays, consumers are more aware of what they eat and also request minimally processed foods and they tend to prefer biodegradable or bio-based packaging over the traditional plastic ones. Therefore, there is an urgent real need to develop newer and safer food packaging systems to improve food shelf-life, whether to reduce food waste (packaged food or the package itself) [1] or to distribute products to more distant places. Furthermore, there is a growing need to provide new solutions to ensure the safety and quality of the packaged foods and products. Due to all these demands, there is a growing market for the development of new packaging solutions, called active packaging (AP), to be applied within several fields, such as pharmaceutical, healthcare or food industries [2]. Active packages are based on the incorporation of active agents with the food packages, thus avoiding the direct addition of chemicals to the food. These active agents can be either incorporated directly in the packaging materials, or be included inside a package as pads, trays, sachets or pouches. These active agents include antioxidants, antimicrobials or absorbers that hand over new properties to the pre-existent material such as oxygen or free radical scavenging (antioxidant/absorber) or microbiological control (antimicrobial) [2]. Over the last decades, active packaging has become a reality for the food packaging industry with the constant growth of the global market for active/intelligent packaging, reaching a compound annual growth rate of 6.9% [3]. There is a vast array of AP solutions currently available in the market; with the vast majority of them having the consumer as the final user and a few of them being intended for business-to-business use (Table **1**).

**Table 1. Examples of worldwide commercially available active packaging solutions.**

| Tradename | Company | AP Type | Materials | Country | Consumers | B2B |
|---|---|---|---|---|---|---|
| **Ageless®** | Mitsubishi Gas Chemical Co. Inc. | Oxygen scavenger | Sachets | Japan | X | |
| **シートドライヤー Sheet dryer** | Torishige | Dessicant | Laminate papers | Japan | X | |
| **EMAP/AMAP** | Perfotec | Modified Atmosphere Packaging (controlled permeability of the packaging and controlled atmosphere) | Plastic trays | The Netherlands | X | X |
| **FreshPaper** | Fenugreen | Fiber-based sheets with organic spices | Paper sheet | USA (also export in Europe) | X | X |
| **Mega$CO_2$** | Pomona | Moisture absorbing pads combined with $CO_2$ scavenger | pads | Poland | X | |
| **EthenAbsorbers/ETENSachets** | Pomona | Ethylene scavenger | sachets | Poland | X | |
| **Oxyguard** | Clariant | Oxygen scavengers | sachets | USA | X | |

*(Table 1) cont.....*

| Tradename | Company | AP Type | Materials | Country | Consumers | B2B |
|---|---|---|---|---|---|---|
| **BreatheWay** | Landec Corporation | Selective permeability films | films | USA | X | |
| **Darex OST** | Darex | Oxygen scavenger | crown | USA | | |
| **OxyFresh** | STANDA Laboratories (EMCO) | $CO_2$ scavenger / $O_2$ emitter | sachets | France | X | |
| **Sanocoat** | Mondi Packaging Flexibles AG | Antimicrobial paper | paper | Austria/Germany | X | |
| **NA** | Erze Ambalaj/Parx Plastics | Antimicrobial tray | plastic trays | Turkey | | X |
| **AntioxidantPack** | BTSA | Antioxidant film | plastic films | Spain | | X |
| **Supasorb** | Thermarite | High capacity moisture absorbing film | pads | Malaysia | X | |
| **Fresh-r-Pax** | Maxwell Chase Technologies LLC | Moisture absorbent | trays, pads, pouches | USA | X | |
| **DriFresh® SeaFresh™ Fresh-Hold** | Sirane | Absorbing pad (moisture/ice/odor) with $CO_2$ emitter | pads | UK, available in Poland | X | |
| **Dri-Fresh® SeaFresh™ Ice-Mats** | Sirane | Seawater-releasing pad to extend seafood shelf-life | pads, mats | UK, available in Poland | X | |
| **Dri-Fresh® Fresh-Hold™ OA** | Sirane | Odour-scavenging pad | pads, labels | UK, available in Poland | X | |
| **Dri-Fresh® Fresh-Hold™ AB** | Sirane | Antibacterial pad | pads | UK, available in Poland | X | |
| **NA** | Artibal S.A. | Antimicrobial coating | films | Spain | X | |
| **NA** | Artibal S.A. | Antioxidant coating | films | Spain | X | |
| **NA** | Goglio SpA | Antioxidant film | films | Italy | X | |
| **NA** | SAMTACK | Antioxidant adhesive for multilayer | adhesive | Spain | X | |
| **Rycoat F-100, Emulactiv C-1** | REPSOL YPF Lubricantes & Especialidades | Antimicrobial/antioxidant coating | paper/cardboard | Spain | X | X |
| **NA** | CellComb | $CO_2$ emitter | pads | Sweden | X | |

These packages have been used to preserve all kinds of foods ranging from more perishable goods, as fresh fruit, vegetables, meat, fish and cheese to more processed foods such as breads, cakes and sweets, sauces and jams, processed and dried meat, snacks and even baby food and pet food. These AP solutions include several absorbers such as moisture and odor absorbers, and ethylene and carbon dioxide scavengers. With respect to antioxidant packaging, the two main types of AP available are the use of oxygen and free radical scavengers. When dealing with antimicrobial packaging, there is a broad array of technologies available aiming at reducing microbial growth in the food product, either by changing the atmosphere (selective permeability films for modified atmosphere and carbon dioxide emitters) or by adding antimicrobial substances ranging from chemicals to natural products such as essential oils or herb extracts. The mode of action of each AP depends on the active agent incorporated and the packaging design as well as the characteristics of the packaged food product (Table **2**).

The emergence and development of these new packaging technologies that interact with food triggered a response from the authorities to ensure their safety towards the consumers. In this regard, the European Union adopted specific

**CHAPTER 2**

# Microplastics and Nanoplastics in Food

**Pilar Fernández-Hernando[1], Rosa Mª Garcinuño-Martínez[1,*]** and **Esther Garrido-Gamarro[2]**

[1] *Departamento de Ciencias Analíticas, Facultad de Ciencias, Universidad Nacional de Educación a Distancia (UNED), Paseo Senda del Rey nº 9, 28040, Madrid, Spain*

[2] *Food Safety and Quality Officer, Fisheries and Aquaculture, Department, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 00153 Roma RM, Italy*

**Abstract:** Plastic production has exponentially increased since the 1950s and reached 322 million tons in 2015. It is expected that the production of microplastic will continue increasing to at least double the production of 2015. As documented in laboratory and field studies, marine organisms of commercial importance for fisheries and aquaculture are affected by microplastics ingestion not only due to the additives used in their manufacture but also because microplastics act as absorbents of persistent organic pollutants (POPs) from the environment. The ingestion of microplastics by aquatic organisms pose a risk to marine environment and food safety. Although microplastics are a human health hazard, their effects on seafood is attenuated by the extraction of the gastrointestinal tract. However, shellfish and other species of crustaceous consumed whole pose a particular concern for human exposure. This chapter discusses the problems associated with microplastics ingested by marine organisms. The most common methods used for sampling, identification, and quantification of microplastics are mentioned and some analytical methods to determine plastic additives and POPs adsorbed on the microplastics in different marine environment matrices are described. Microplastic dietary intake and the limitations for food safety risk assessment are also addressed. Since 2004, many types of research have focused on this topic and analyzed microplastics in various environmental matrices. However, the development of standardized methods for the screening, identification, detection, and quantification of microplastics in marine environment remains a challenge.

**Keywords:** Additives, Analytical methods, Environmental matrices, Food safety, Microplastics, Risk assessment, Seafood.

## INTRODUCTION

Today, plastics have become one of the most utilized materials worldwide, being

* **Corresponding author Rosa Mª Garcinuño-Martínez:** Departamento de Ciencias Analíticas, Facultad de Ciencias, Universidad Nacional de Educación a Distancia (UNED), Paseo Senda del Rey nº 9, 28040, Madrid, Spain; Tel: +34913987366; E-mail: rmgarcinuno@ccia.uned.es

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

an essential constituent of daily life items.

Since their first development in the 1800s, plastics production has suffered changes to meet the needs of a variety of sectors and consumers and has enabled technological improvements and solutions. Due to their functional properties, plastics displaced traditional materials. Plastics are a range of synthetic or semi-synthetic materials delivered from fossil resources and organic products and are usually divided into three categories: thermoplastics (polymers that can be remelted), thermosets (polymers that remain in a permanent solid state once hardened) and elastomers (elastic polymers that return to their original shape). Depending on the intended use of the plastic, polymers with different physical and chemical properties can be mixed among them and with additives such as plasticizers, flame-retardants, colorants, and antioxidants to enhance plastic performance, which can complicate recycling and the evaluation of their impact on the environment and on human health. The increasing production of plastic requires efficient waste management systems that few countries can implement. For this reason, it is estimated that most plastics persist in the environment whole or fragmented, contributing to plastics and microplastics pollution. Microplastics can become a food safety threat when they get into the food chain and have been found in a variety of food commodities such as salt, beer, honey or fish. Seafood is the best-studied species concerning microplastic intake.

## MICROPLASTICS AND NANOPLASTICS DEFINITION

There is an ongoing debate about what can be considered microplastics and nanoplastics. One of the most acknowledged definitions describes microplastics as plastic particles composed of a heterogeneous mixture of different shaped materials in the range of 0.1-5000 μm [1, 2]. Nanoplastics are identified as plastic particles whose size is ranging from 0.001 μm to 0.1 μm [3]. Although the size of microplastics is an important factor that determines their impact in the living organisms that ingest them, shape might also be an influencing factor. There are two types of plastics based on how they are produced or generated. Primary microplastics are produced for industrial purposes such as plastic manufacture or cosmetics.

Secondary microplastics are generated by weathering processes and fragmentation of larger plastics [4], which may occur when plastics are disposed of in the environment or when using plastic products such as textiles or tires. Eventually, both types of microplastics will end up polluting the environment and entering food supply chains.

## MICROPLASTICS AND NANOPLASTICS COMPOSITION

As mentioned above, plasticizers, flame-retardants or antioxidants are used as additives in plastics. When plastics reach the environment, they can adsorb or absorb contaminants from the surroundings, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated pesticides, which are considered persistent organic pollutants (POPs), trace metals, and microorganisms such as pathogenic bacteria or viruses [5].

### Polymers

Monomers, such as ethylene, propylene, and styrene are used as building blocks of polymers that lead to the production of a variety of materials. The most commonly used polymers are: acrylonitrile butadiene styrene (ABS), acrylic (AC), epoxy resin (EP), expanded polystyrene (EPS), polyethylene high density (HDPE), polyethylene low density (LDPE), polyethylene linear low density (LLDPE), polyamide (Nylon) 4, 6, 11, 66 (PA), polycarbonate (PC), polycaprolactone (PCL), polyethylene (PE), polyethylene terephthalate (PET), poly (glycolic) acid (PGA), poly(lactide) (PLA), poly(methyl methacrylate) (PMMA), polypropylene (PP), polystyrene (PS), polyurethane (PU), polyvinyl alcohol (PVA), polyvinyl chloride (PVC), styrene-butadiene rubber (SBR) and thermoplastic polyurethane (TPU) [5]. All these polymers can be expected in microplastic pollution and therefore get into different food chains.

### Flame-Retardants

At present, more than 175 chemicals are classified as flame-retardants (FRs) [6]. These compounds are commonly added to polymers to reduce the flammability of plastics and some are not normally added to polymers in processing, but can be found in a polymer matrix from leaching out of the contents. Brominated flame retardants contain a wide variety of organic compounds including polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) that are the most used chemicals in the manufacture of plastic. They are commonly added to polystyrene, polyesters, polyolefins, polyamides, epoxies, and ABS. HBCDs and PBDEs are used by simple blending with the polymers, therefore these compounds are most likely to leach out of the final products [7]. This poses an environmental and food safety concern because PBDEs and HBCD are considered POPs by the Stockholm Convention and many studies associate them with endocrine disorders, teratogenicity, and kidney and liver toxicity [8, 9].

### Plasticizers

Substances such as phthalates and bisphenols (BPs) are used as plasticizers,

CHAPTER 3

# Nanotechnology in the Food Field: Application of Metal-Based Nanoparticles

**Beatriz Gómez-Gómez** and **Yolanda Madrid***

*Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040, Madrid, Spain*

**Abstract:** Nanotechnology offers a wide range of applications in the food sector such as development of new tastes and textures, nanoencapsulation of bioactive food components, design of nutrient delivery systems, nanosensors to detect spoilage or contamination, and the design of new food packaging materials. Although metal-based nanoparticles (AgNPs, $SiO_2$NPs, $TiO_2$NPs, ZnONPs...) have extensively been applied due to their antimicrobial, antioxidant and UV-blocking properties, there is limited knowledge about the impact of nanoparticles on human health and environment. For safety reasons, the EU has issued regulations requiring labelling of the nanomaterials in the ingredients list. Therefore, new analytical methods should be used to characterize nanomaterials but, since there is no single and universal method that can be applied to fully characterize nanoparticles, the need for multimethod approaches is widely acknowledged. This chapter focuses primarily on the application of metal-based nanoparticles in the food sector and the analytical methodologies used for nanoparticle characterization. Regarding the applications of nanoparticles, special attention should be paid to their antimicrobial properties and their use for developing active food packaging materials. Since the characterization of nanoparticles in complex matrices is troublesome, a detailed description of the prospects and difficulties of the analytical techniques commonly employed is given. Similarly, factors affecting nanoparticles stability such as sample preparation, interaction with food matrices, food stimulants, and chemicals used in "in vitro" gastric digestion procedures are also described. Finally, EU regulatory guidelines on nanomaterials are included and discussed.

**Keywords:** Analytical methodologies, Current EU directives, Food, Metal-based nanoparticles, Nanoparticles stability, Nanotechnology, Sample treatment.

## INTRODUCTION

Nanotechnology has a huge impact on our daily life. It has revolutionized the industrial sector due to large-scale production of nanosized materials and the

* **Corresponding author Yolanda Madrid:** Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040, Madrid, Spain; Tel/Fax: +34 913945149; E-mail: ymadrid@quim.ucm.es

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

growing investment in this field from governments and industry worldwide. According to the European Commission recommendation, a nanomaterial can be defined as "*a natural, incidental, or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1-100 nm. In specific cases and where warranted by concerns for the environment, health, safety, or competitiveness, the number size distribution threshold of 50% may be replaced by a threshold between 1% and 50%*" [1]. The impact of nanotechnology is such that the term "nanotechnology" has become synonymous with promising innovative products.

Nanosized materials have gained special attention due to their singular properties compared to bulk materials. First, they have a greater surface area per unit mass in contrast to larger particles that makes them more reactive. Second, the dominance of quantum effects at the nanoscale significantly affects the optical, magnetic, and electrical properties of the material. Among nanostructures, nanoparticles (NPs) are a category with important applications on sectors like medicine and medical devices, engineering and communication technologies, and in some industrial areas such as electronics, photonics, textile, pharmaceutical, food, and cosmetics. A nanoparticle is defined as a nanoform that has one or more dimensions of the order of 100 nm or less [2, 3]. This group comprises a heterogeneous variety of materials that are classified based on their composition into different categories: carbon-based nanoparticles (nanotubes, fullerenes, NPs of latex and graphenes), metal/metalloid-based nanoparticles (Fe, Au, Ag, $TiO_2$NPs, ZnONPs, $SiO_2$NPs, $CeO_2$NPs, quantum dots) and aluminium silicates (zeolites, clays). Nowadays, there are more than 1600 nanotechnology-based consumer products on the market [4]. Among all, metal and metal oxide nanoparticles have gained great research attention due to their relevant antioxidant, antimicrobial and blocking UV properties.

The use of nanomaterials in the food sector has exponentially grown in recent years. Nanotechnology offers a wide range of applications (Fig. **1**) in food processing such as the development of new tastes and textures, and the encapsulation of food components and additives to control the release of flavors and deliver nutraceuticals. Nanomaterials in the food industry have been mostly applied to develop food packaging materials for extending the shelf-life of foodstuffs. Nanoparticles are added to the packaging materials to improve their mechanical strength and barrier properties. Moreover, smart and intelligent food packages including nanoparticles as active components and nanosensors have been manufactured for food quality parameters control such as moisture, oxygen and carbon dioxide contents, microbial surface contamination, freshness and food conditions during transport and storage. Although most of the aforesaid

applications are still in progress, the prospects of nanotechnology in the food technology sector are extraordinary.

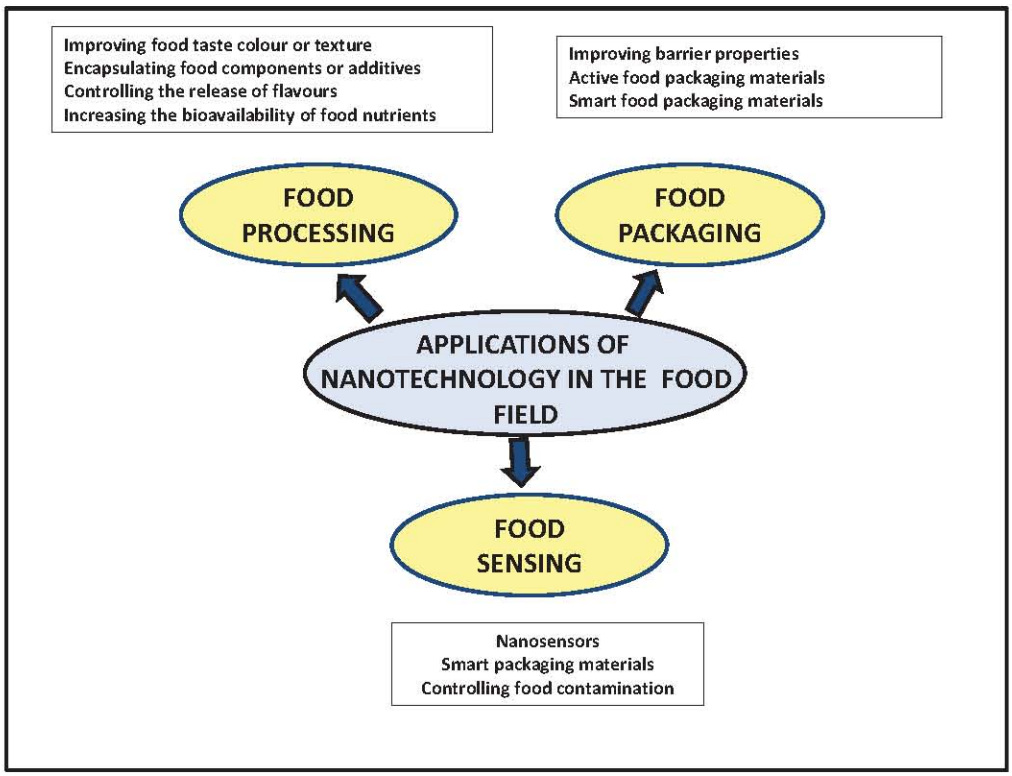


**Fig. (1).** Main applications of nanotechnology in the food field.

However, the widespread use of nanotechnology in our daily life is a matter of concern due to the increasing exposure of humans and ecosystems to nanoparticles which makes necessary a thorough assessment of their toxicological impact. For safety reasons, the EU has issued regulations requiring labelling of the nanomaterials in the ingredients list [5]. Consequently, there is an urgent need for developing analytical methods to identify nanoparticles in consumer products and to enable scientists to detect, identify and quantify them in complex matrices such as food and environmental samples. Unfortunately, since there is no single and universal method that can be applied to fully characterize nanoparticles, the need for multimethod approaches is widely accepted. The most common techniques for characterizing nanoparticles are Dynamic Light Scattering (DLS), Multi-angle Light Scattering (MALS) and nanoparticles tracking analysis (NTA); classical electron microscopy (TEM/SEM); analytical separation techniques such

CHAPTER 4

# Halogenated and Organophosphorus Flame Retardants

**Òscar Aznar-Alemany** and **Ethel Eljarrat***

*Institute of Environmental Assessment and Water Research, Spanish National Research Council (IDAEA-CSIC), C/ Jordi Girona, 18-26. 08034 Barcelona, Spain*

**Abstract:** Flame retardants are applied to a wide range of materials to improve their fire resistance. However, they leak from those materials into the environment. There are many compounds used as flame retardants, the most relevant organic ones being polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), other halogenated flame retardants (HFRs) and organophosphorus flame retardants (OPFRs). Exposition to flame retardants can be through ingestion, inhalation and skin permeation. Different studies report that food account for most of the exposition to PBDEs. Data indicates that seafood is the main contributor to PBDE intake in Europe and Japan, while meat is the main contributor in the United States and Canada. For this reason, it is one of the main public health interests that food be innocuous. This chapter compares seventeen publications that apply methods suitable for the analysis of flame retardants in food. Some publications include different methods targeting different groups of compounds. PBDEs and most HFRs are commonly analyzed together by GC. HBCD tends to be extracted separately and analyzed by LC. OPFRs are also extracted and analyzed independently, but few methods target them currently. The present text presents and compares the sample treatment, the instrumental analysis and the quality parameters for the listed methods. A final comment on levels of flame retardants in food and dietary intake is provided.

**Keywords:** BFRs, Clean-up, Dechloranes, Dietary intake, Extraction techniques, Flame retardants, Food analysis, Food safety, Gas chromatography, HBCD, HFRs, Instrumental analysis, Lipid removal, Liquid chromatography, Mass spectrometry, Methods comparison, OPFRs, PBDEs, Quality parameters, Sample treatment.

## INTRODUCTION

Flame retardants (FRs) are a group of compounds that are added to materials to

* **Corresponding author Ethel Eljarrat:** Institute of Environmental Assessment and Water Research, Spanish National Research Council (IDAEA-CSIC), C/ Jordi Girona, 18-26. 08034 Barcelona, Spain; Tel: +34 934006100 (extension 1627); E-mail: eeeqam@cid.csic.es

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

increase their resistance to fire. The search for flame retarding compounds produced, among others, halogenated flame retardants (HFRs), mainly brominated and chlorinated.

In the gas phase, HFRs capture hydroxyl and hydrogen radicals produced in the first steps of combustion and which allows the propagation of the reaction [1].

At the beginning of the 20$^{th}$ century, the electrical industry needed a dielectric insulator that acted as a FR. Production of polychlorinated biphenyls (PCBs) started in the United States in 1929, then in Europe and later in Japan in 1954. A Swedish biologist detected PCBs in fish in 1966 [2]. Two years later, a thousand of Japanese people were found intoxicated with PCB-contaminated rice oil. PCBs were banned in Japan in 1972 and their production in the United States was stopped in 1976 [3].

Hexacyclopentadiene was described first in 1930 and was later considered as an insecticide and FR [4]. Hooker Electrochemical commercialized its dimer, mirex, in the 1960s calling it Dechlorane. However, mirex was banned in 1977 due to its degradation forming a carcinogenic compound [3]. In 1964 Hooker Electrochemical -currently Occidental Chemical (OxyChem)- had already developed a derivate from hexacyclopentadiene which they named Dechlorane Plus.

Some brominated flame retardants (BFRs) available in the 1950s were pentabrominated diphenyl ether (pentaBDE), tris(2,3-dibromopropyl) phosphate (Tris) and tetrabromobisphenol A (TBBPA). As BFRs were more effective than chlorinated FRs, BFRs allowed for smaller amount of additives in the materials, thus not compromising so much their physical properties and BFRs became popular very fast. On the other hand, some of them had to be banned do to their toxicity, as was the case of Tris in 1977 [3].

HFRs are used for years until the scientific community gathers enough data to assess their adequacy. Being persistent, the impact of banned HFRs on the environment and the organisms can last long after their ban.

Not all FRs are halogenated; there are also organophosphorus flame retardants (OPFRs). OPFRs accounted for 20% of the FRs used in Europe in 2006 —which doubles the amount of PBDEs used that year— and have been increasingly applied after the restrictions on PBDEs [5]. Apart from FRs, OPFRs are applied as plasticizers as well.

The fact that FRs are found in the environment and accumulate in organisms, some of which serve to feed humans, implies that these contaminants are likely to be present in food to some extent. Humans are at the top of the food web and,

thus, the final recipients of the biomagnification effect.

Exposition to FRs can be through ingestion, inhalation and skin permeation. A study performed in Vietnam considering different types of exposition to polybrominated diphenyl ethers (PBDEs) estimated that fish consumption accounted for 70% of the total exposition to PBDEs and 80% of the exposition to BDE-209 [6]. Some preliminary studies in all kinds of foods from Canada, Japan, the United States of America and Europe concluded that the average daily intake of PBDEs was between 13 and 113 ng $day^{-1}$ [7]. Their data also showed that seafood is the main contributor to PBDE intake in Europe and Japan, while meat is the main contributor in the United States and Canada. The average daily intake of PBDEs per kilogram of body weight in Europe was 2.2 ng $bw^{-1}$ $day^{-1}$. A Swedish study calculated that fish accounted for more than 60% of the total intake of PBDEs and more than 80% of the intake of BDE-47 [8].

Seafood production has grown to 3.2% every year since 1961 [9]. Nowadays, aquaculture provides half the seafood consumed worldwide. On average, a person consumes 20 kg of fish per year and 17% of the world intake of protein comes from fish.

Not only do organisms accumulate contaminants present in the environment, but the feed used in farms and fish farms contains animal parts with no commercial value, which might add their accumulated contaminants into the diet of new animals.

For this reason, it is one of the main public health interests that food —and especially seafood be innocuous.

## FLAME RETARDANTS

### Polybrominated Diphenyl Ethers and Hexabromocyclododecane

PBDEs and hexabromocyclododecane (HBCD) are some of the most popular FRs. They can be found in a broad variety of elements such as plastics, furniture, vehicles and electronic appliances [10].

There are 209 PBDE congeners depending on their degree of bromination and the position of the bromine atoms (Fig. **1**). As their structures are analogous to those of PCBs, the same nomenclature by Ballschmiter and Zell is used [11].

The three commercial mixtures are PentaBDE (0-1% triBDE, 24-37% tetraBDE, 50-60% pentaBDE and 4-8% hexaBDE), OctaBDE (10-12% hexaBDE, 43-44% heptaBDE, 31-35% octaBDE, 10-11% nonaBDE and 0-1% decaBDE) and

CHAPTER 5

# Dioxins and PCBs in Food and Feed Matrices: Advances in Physico-Chemical Methods and EU Regulatory Framework

**Jordi Parera, Manuela Ábalos** and **Esteban Abad***

*Laboratorio de Dioxinas, Departamento de Química Ambiental, IDAEA-CSIC, C/ Jordi Girona, 18-26, 08034 Barcelona, Spain*

**Abstract:** Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are major representatives of persistent organic pollutants. While PCDD/Fs are unwanted by-products, mainly from waste incineration and industrial processes, PCBs were manufactured and widely used as transformer oils until bans enter in force at the late '70s. These compounds are highly toxic and can easily bioaccumulate and biomagnify throughout the food chain reaching the top living organisms, including human beings. Food is the main route of human exposure to PCDD/Fs and PCBs, with products from animal origin contributing largely to the dietary intake. In this sense, several contamination episodes involving feed and food products that occurred at the late '90s led to the establishment of a European regulatory framework that aims to both, set maximum levels for these compounds in different food/feed categories and to lay down analytical methods for the determination of these compounds. In this work, an overview of the different chemical methodologies that have been applied during the last decades to the determination of PCDD/Fs and PCBs, more in particular dioxin-like PCBs, in food and feed samples is presented. Advances in extraction and purification steps are described, but special attention is given to the evaluation of several mass spectrometric techniques in comparison to gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS), which has traditionally been the unique confirmatory technique until recently.

**Keywords:** Clean-up, Dioxin-like PCBs, EU regulations, Extraction, Feed, Food, GC-HRMS, GC-MS/MS, Ion trap, Mass spectrometry, PCBs, PCDDs, PCDFs, Triple quadrupole.

* **Corresponding author Esteban Abad:** Laboratorio de Dioxinas, Departamento de Química Ambiental, IDAEA-CSIC, C/ Jordi Girona, 18-26, 08034 Barcelona, Spain; Tel: +34 934006185; E-mail: esteban.abad@idaea.csic.es

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

## INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two families of compounds that comprise a total of 210 different congeners (*i.e.*, 75 PCDDs and 135 PCDFs).

They are also commonly known as dioxins and furans, respectively, or only as dioxins. Their molecule is characterized by two benzene rings connected by one oxygen atom, in the case of furans, or two oxygen atoms, in the case of dioxins. Each one of the benzene rings can have several chlorine atoms; therefore a total of eight homologues groups can be defined taking into account the degree of chlorination, from one chlorine atom present (mono-) to a maximum of eight chlorine atoms (octa-). This particular molecular structure presents high stability; in consequence, dioxins are characterized by their resistance to chemical and biological degradation.

Dioxins were first identified in fly ash and gas emissions from incinerator facilities at the end of the '70s [1]. Nowadays, it is well-known that these compounds are unwanted by-products mainly originated from anthropogenic activities, being the major source of waste incineration and some specific industrial processes (*e.g.*, cement kilns, pulp and paper mills, sintering plants or pesticide manufacturing). There are also diffuse sources, such as vehicle exhaust [2, 3].

The toxic effects of PCDD/Fs to the living organisms, and in particular to humans, have been widely studied. It has been demonstrated that they act as endocrine disruptors and are associated with cancer risk [4, 5]. However, it has to be remarked that among the 210 PCDD/F congeners, only those with chlorine atoms at least in the 2,3,7,8 positions of the molecule have been found to show toxicological activity. This way, the number of target congeners to be considered for analysis is reduced to 17 (*i.e.*, 7 PCDDs and 10 PCDFs).

On the other hand, polychlorinated biphenyls (PCBs) are another family of compounds, which includes a total of 209 congeners. For these compounds, the degree of chlorination varies from one (mono-) to ten (deca-) chlorine atoms present at the molecule. Contrary to what it has been mentioned about PCDD/F sources, PCBs were manufactured and commercialized in 1929, mainly as electrical insulating fluids, and they were widely used until restrictions to their production and application came into force between the end of the '70s and the beginning of the '80s [6]. Among the 209 PCB congeners, those without chlorine atoms in the *ortho* position of the molecule (*i.e.*, 4 non-*ortho* PCBs) and those with only one chlorine atom in this position (*i.e.*, 8 mono-*ortho* PCBs) show similar physico-chemical characteristics and toxicological activity to dioxins.

These 12 PCBs are the so-called dioxin-like PCBs (dl-PCBs).

Due to the extreme stability of dioxins and PCBs, they are highly persistent once they have been released in the environment and can be transported over long distances. Besides, the lipophilic character of these compounds leads them to bioaccumulate and to biomagnify throughout the food chain reaching the top living organisms, including human beings. All these particularities, together with their proved high toxicity even at very low concentrations (trace levels), have placed dioxins and PCBs among the most representative persistent organic pollutants (POPs).

The toxic effects of dioxin-like compounds are related to the interaction with the aryl hydrocarbon receptor (AhR) on the cells [7]. Although all toxic congeners present a similar mechanism of interaction, the toxicological potential varies depending on the degree of chlorination and the distribution of the chlorine atoms at the molecule. In general, for PCDD/Fs, toxicity decreases when the number of chlorine atoms increases, being 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) the most toxic dioxin congener. Taking this into account, a methodology based on the assignment of relative value, defined as the toxic equivalency factor (TEF), to each dioxin-like compound has been proposed. The individual TEFs are related to the maximum value assigned to the 2,3,7,8-TCDD (TEF=1), this way the toxicity of a sample due to the presence of PCDD/Fs and dl-PCBs can be calculated as the sum of the products obtained by multiplying the concentration of each congener by its corresponding TEF. This final result (sum) is known as the total toxic equivalent (TEQ) and allows the comparison of samples in terms of their toxicological potential.

Several TEF schemes have been established with slight differences in the assigned TEF values. In particular, panel of expert of the World Health Organization (WHO) established consensual TEFs for dioxins and dl-PCBs for human, fish and wildlife risk assessment in 1997 [8]; later on, in 2005, these values were updated after a first re-evaluation [9]. Table **1** shows a comparison between the first WHO-TEF values assigned (1998) and the revised ones (2005). The WHO-TEF scheme is important since it was the one adopted by the end of the '90s to set tolerable weekly/daily/monthly intakes. Moreover, this scheme has later been used to establish maximum levels, expressed in TEQ, for PCDD/Fs and dl-PCBs in several food and feed products.

Levels of dioxins and PCBs increased dramatically until the late '70s or early '80s, due to the lack of knowledge about their presence in the environment and the adverse effects of these compounds for the living organisms. Since then, significant efforts have been made to adopt strategies to reduce unintentional

CHAPTER 6

# Pesticides

**Vicente Andreu** and **Yolanda Picó***

*Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV), Desertification Research Center (CIDE), Joint Research Center CSIC-UV-GV, Moncada, Spain*

**Abstract:** Analysis of pesticide residues is very important to enforce legislation and guarantee food safety. The correct use of pesticides is still crucial in agriculture because they provide spectacular increases in crop yields and ensure global demand for grain. However, the indiscriminate, incorrect and/or excessive use of pesticides in agriculture may have some serious adverse effects such as the accumulation of residues in food. Pesticide residues are controlled worldwide by maximal residues limits (MRLs), not the same in all countries but generally ranging from a few μg $kg^{-1}$ (usually for pesticides that are banned) to a few tens of mg $kg^{-1}$. Determining pesticides at this concentration requires sensitive, accurate and robust instrumentation, and trained personnel as well. This chapter explores the latest advances to determine pesticide residues as accurately as possible in the shortest time. A description of aspects like improvement of high-throughput methods specificity and advances in the determination by gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) or (bio)sensors, are presented in this chapter. The focus is on multi-residue o multiplexed analysis that will offer rapidity and economy in order to achieve the required sensitivity (<0.01 mg $kg^{-1}$). The primary purpose of this chapter is to provide the reader with a state- of- the- art assessment and identification of gaps within this field, and to establish future trends in the extraction, purification, and determination of pesticide residues.

**Keywords:** Dispersive liquid-liquid extraction, Food of animal origin, Fruits and vegetables, Gas chromatography-mass spectrometry, Liquid chromatography-mass spectrometry, QuEChERS, QuPPe, Sensors.

## INTRODUCTION

Pesticides are a group of just a few natural molecules and a majority of synthetic ones developed to eliminate pests in both agriculture and livestock. To give

* **Corresponding author Yolanda Picó:** Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV), Desertification Research Centre (CIDE), Joint Research Centre CSIC-UV-GV, Moncada-Naquera Road Km 4.5, 46113 Moncada, Spain; Tel: +34 963424215; E-mail: Yolanda.Pico@uv.es

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

an idea of the number of this type of compounds, the reare approximately 1000 active ingredients which have no chemical relationship among them and cover a wide range of physico-chemical properties.

Pesticides play a significant role in food production because they increase crop yields and the number of harvests that can be obtained per year. This is particularly important in countries with endemic food shortages that may expect an exponential growth of population in the coming years. However, pesticides are significantly toxic to biota and people. Toxicity depends on their function, action mechanism, doses, and exposure time. Several studies indicate that dietary exposure to pesticides (or long-term exposure) is associated with a broad spectrum of adverse effects on the development and reproduction of human beings, and on their nervous and immune systems. Pesticides can also cause oxidative stress, cell damage, endocrine disruption and different types of cancer.

The production, distribution and application of pesticides is strictly regulated and subject to a tight control due to their toxicity. Since pesticides are purposely applied on large surfaces, their exact destination is difficult to control. Monitoring their presence in food to ensure food safety is also crucial.

Analytical laboratories aim to detect, identify and quantitate many different pesticides with diverse physical-chemical properties in increasingly complex matrices at trace concentrations (ca. 10 $\mu g\ kg^{-1}$).

This chapter outlines the basic principles of advanced extraction and determination approaches, their advantages and drawbacks, the suitability of analytical validation parameters and their robustness and usefulness for pesticide residue determination.

## LEGISLATION

The legislation regulating pesticide residues in food is very extensive. Only specifically authorized pesticides may be used, and pesticide levels in food must be below the established maximal residues limits (MRLs) to be apt for consumption. Additionally, the guidelines concerning the analytical methods used to determine these residues are also very strict.

The first guideline marks the number of substances to be determined. There are about 1000 active substances considered as pesticides but important differences exist in the authorized products among countries. The European Union (EU) reduced in 2008 the number of authorized substances. However, this is only relatively useful since markets are global and products banned in the European Union are authorized in other parts of the world. Therefore, at least in theory, the

nearly 1000 active substances that exist need to be monitored.

The second guideline establishes the limits of detection to be reached by analytical methods. Residues are the rest of the pesticide formulations that remain on or in the plant after application. MRLs are defined by the EU [1] as "the upper legal levels of a concentration for pesticide residues (expressed in mg $kg^{-1}$) in or on food or feed based on good agricultural practices (GAP) and to ensure the lowest possible consumer exposure". MRLs for different crops and pesticides, as established in the EU, can be found in the MRL database on the Commission website [1]. If a pesticide is not regulated within the EU, the MRL applied is 0.01 mg $kg^{-1}$. It is assumed that this value is the limit of detection achievable using state-of-the-art instrumentation. "Pesticide residues" according to the EU include conversion and degradation products, metabolites, reaction products, and impurities that have toxicological or environmental significance [1]. This is what makes these compounds determination more complicated, because it is mandatory to identify the pesticide and a variable number of its metabolites.

The EU legislation does not establish official analytical methods for the determination of pesticide residues in food. However, although their use is not mandatory, standardization agencies propose a number of well-validated methods that could be used. Instead, the EU has approved guidelines establishing the minimum quality requirements of an analytical method to be applied. The main problem with these analyses is that pesticides are at a very low concentration in the samples, and therefore, determination has to be precise and accurate and reach a very high sensitivity. The most important guidelines applicable to pesticide residues can be accessed on the EU website.

## ANALYTICAL METHODS

Modern scientific methods to measure pesticide residues in plants involved three different phases that can be summarized as: 1) Sampling, and sample preparation, 2) Extraction and clean-up of the extract, 3) Determination. Fig. **(1)** schematizes the workflow of these methods as well as the time spent in each step.

Sample and sample preparation involve a series of well-known procedures that are outside of the scope of this chapter. Sampling procedure to determine pesticides in food is well established in the EU guidelines. Sample preparation involves cutting, chopping and homogenization. Samples are commonly preserved frozen at -20 °C.

The extraction and clean-up procedures are commonly determined by: (i) the characteristics of the matrix (% of proteins, lipids and carbohydrates, presence of salts, *etc.*), (ii) type of pesticides to be determined, and (iii) determination

CHAPTER 7

# Perfluoroalkyl Substances (PFASs) in Foodstuffs and Human Dietary Exposure

**Qian Wu** and **Kurunthachalam Kannan***

*Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, University at Albany, State University of New York, Albany, NY 12201, USA*

**Abstract:** Perfluoroalkyl substances (PFASs) have been used as surfactants and surface protectors in many industrial materials and consumer products. PFASs have been reported to be associated with numerous adverse health outcomes in humans. Americans have the highest levels of PFASs in their bodies in comparison with populations from other countries. To our knowledge, data on the sources and pathways of human exposure to PFASs are limited. In this study, we determined PFASs in a wide variety of samples (water, food, indoor dust), and calculated exposure dose from various environmental sources including diet. A mass balance analysis was performed by comparison of calculated exposure doses (environmental sources) with modeled doses (biomonitoring results). PFASs occurred widely in drinking water, food, and indoor dust. Breast milk is the major source of exposure to PFASs in breast-fed infants. For PFOS and PFOA, indoor dust and diet are the major sources of exposure in adults. The results of mass balance analysis showed a good agreement between exposure doses calculated based on external sources and those modeled from biomonitoring studies.

**Keywords:** Drinking water, Exposure assessment, biomonitoring, Foodstuffs, Perfluoroalkyl substances, PFASs.

## INTRODUCTION

### Sources of Human Exposure to PFASs

Perfluoroalkyl substances (PFASs) are a class of man-made chemicals, with a fully fluorinated hydrocarbon chain (tail) and a hydrophilic functional group (head). The fluorinated hydrocarbon moiety is both lipophobic and hydrophobic. Due to this unique property, PFASs have been used as surface protectors and surfactants in many industrial applications and consumer products such as textiles,

* **Corresponding author Kurunthachalam Kannan:** Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, University at Albany, State University of New York, Albany, NY 12201, USA; Tel: +1-518-474-0015; E-mail: kurunthachalam.kannan@health.ny.gov

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

leathers, waterproof clothing, carpets, specialty papers including food packing, cleaning agents, floor polishes, fire-fighting foams, and insecticides [1]. Two processes have been used for the production of PFASs: electrochemical fluorination (ECF) that produces a mixture of linear and branched isomers; and telomerization process that produces only linear products. Perfluorooctanesulfonyl fluoride (POSF) is a product of ECF and is a precursor to produce POSF-based PFASs such as perflurooctanesulfonate (PFOS). PFOS can also be metabolized from many other POSF-based compounds including perfluorooctane sulfonamide (PFOSA) and perfluorooctane sulfonamidoalcohols, which are often referred to as "precursors". PFOA and its salts are also produced by telomerization process. PFOA is an emulsifier in the production of fluoropolymers and fluoroelastomers, and is also a degradation product of fluorotelomer alcohols (FTOHs; also referred to as "precursors of PFOA"). Fluoropolymers are used in various applications including construction, automobile, electronics, telecommunication, non-stick coating, thread sealant tape, and breathable clothing, for their stability, strength, and durability. FTOHs are used in surfactants and surface protectors, in carpets, textiles, painting, papers, non-stick cookware coating, and fire-fighting foams. It is estimated that over 4000 per- and polyfluoroalkyl substances are, or have been, on the global market [2], but only a limited number of PFASs including PFOS and PFOA have been studied for over the past two decades due their predominance in environmental and biological samples [3]. The chemical formulae for the most commonly studied perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs) and their major precursor compounds are listed in Table **1**.

**Table 1. Chemical formula of commonly studied PFASs and their precursors.**

| Name | Abbreviation | Formula |
|---|---|---|
| Perfluorobutanesulfonate | PFBS | $C_4F_9SO_3^-$ |
| Perfluorohexanesulfonate | PFHxS | $C_6F_{13}SO_3^-$ |
| Perfluorooctanesulfonate | PFOS | $C_8F_{17}SO_3^-$ |
| Perflorodecanesulfonate | PFDS | $C_{10}F_{21}SO_3^-$ |
| Perfluorooctane sulfonamide | PFOSA | $C_8F_{17}SO_2NH_2^-$ |
| Perfluorooctanesulfonyl fluoride | POSF | $C_8F_{17}SO_2F$ |
| N-methyl perfluorooctane sulfonamidoethanol | N-MeFOSE | $C_{11}H_8F_{17}NO_3S$ |
| N-ethyl perfluorooctane sulfonamidoethanol | N-EtFOSE | $C_{12}H_{10}F_{17}NO_3S$ |
| N-methyl perfluorooctane sulfonamido ethylacrylate | N-MeFOSA | $C_{14}H_{10}F_{17}NO_4S$ |
| N-ethyl perfluorooctane sulfonamido ethylacrylate | N-EtFOSA | $C_{15}H_{12}F_{17}NO_4S$ |
| Perfluorohexanoic acid | PFHxA | $C_5F_{11}COOH$ |
| Perfluoroheptanoic acid | PFHpA | $C_6F_{13}COOH$ |

*(Table 1) cont.....*

| Name | Abbreviation | Formula |
|---|---|---|
| Perfluorooctanoic acid | PFOA | $C_7F_{15}COOH$ |
| Perfluorononanoic acid | PFNA | $C_8F_{17}COOH$ |
| Perfluorodecanoic acid | PFDA | $C_9F_{19}COOH$ |
| Perfluoroundecanoic acid | PFUnDA | $C_{10}F_{21}COOH$ |
| Perfluorododecanoic acid | PFDoDA | $C_{11}F_{23}COOH$ |
| 4:2 Fluorotelomer alcohol | 4:2 FTOH | $C_4H_5F_9O$ |
| 6:2 Fluorotelomer alcohol | 6:2 FTOH | $C_8H_5F_{13}O$ |
| 8:2 Fluorotelomer alcohol | 8:2 FTOH | $C_{10}H_5F_{17}O$ |
| 10:2 Fluorotelomer alcohol | 10:2 FTOH | $C_{12}H_5F_{21}O$ |

In 2001, following the discovery of global distribution of PFASs [3], the 3M Company, the major manufacturer of PFOS-based chemistries, announced phase-out of ECF production of all POSF-based compounds in the U.S. However, the production of PFOS continued in Europe, Japan, and China [4]. The production of PFOA by telomerization process, by other manufactures continued [5]. Following the phase-out of POSF-based chemistry by 3M Company in 2001, the global production of PFASs was thought to be dropped. In May 2009, PFOS and its salts were listed under the Stockholm Convention as Persistent Organic Pollutants (POPs) for their persistent, bioaccumulative, toxic (PBT), and long-range transportation properties [6]. Currently, China is reported to continue the production POSF based compounds.

PFOS and PFOA are bioaccumulative and, PFOS particularly can biomagnify in the food chain. PFOS has been detected at higher concentrations in top predators (bald eagles, dolphins and polar bears) than in animals at the lower trophic levels in the food chain [3, 7]. PFOA is more frequently detected in aquatic medium (*e.g.*, water) than that of PFOS, and PFOA is relatively more water soluble than PFOS [8, 9]. Several precursors of PFASs such as perfluoroalkyl sulfonamides and FTOHs have been reported to be transformed into perfluorinated acids in biological and environmental media [10, 11]. Several PFASs, especially PFOS and PFOA, have been detected globally in air, water, soil, fish, birds, marine mammals, and humans [3, 11 - 16].

Diet has been suggested as an important source of PFAS exposures in humans. Following the release into the environment, PFASs can concentrate and accumulate in plants and animals at the bottom of the food chain, which are then consumed by animals at the higher trophic levels [7, 17, 18]. One of the main sources of PFASs to humans is food producing animals and plants. PFASs were reported to occur in drinking water [19 - 23]. Discharge of wastewater has been

**CHAPTER 8**

# Mercury

**Zoyne Pedrero Zayas***

*CNRS/UNIV PAU & PAYS ADOUR, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux, UMR 5254, 64000, Pau, France*

**Abstract:** Mercury (Hg) pollution is an acknowledged major environmental problem. Considering its extreme toxicity, Hg has recently been included in the top ten list of chemicals of major public health concern according to the World Health Organization. Once released into the environment, it is transformed in aquatic ecosystems by microorganisms into the neurotoxic methylmercury. The hazardous effect is then biomagnified through the trophic/food chain. Diet is considered the main exposure pathway of Hg in humans. Therefore, safety values have been established by food safety authorities in order to protect consumers. Seafood, followed by rice, is the primary source of Hg in the human diet. A variety of analytical methodologies are available for the analysis of Hg and its species in food. This chapter presents recent advances in the determination of Hg in foodstuffs. Special attention is given to innovative Hg (species) extraction and preconcentration systems assisted by nanoparticles. Non-chromatographic approaches, as an alternative to classical chromatographic approaches used for speciation are detailed. The potential and limitations of Hg isotopic analysis in food are also discussed.

**Keywords:** Certified reference materials, Diet, Fish, Food, GC, HPLC, ICP-MS, Isotopic dilution analysis, Isotopic fractionation, MC-ICP-MS, Methylmercury, Mercury, Mercury species, Non-chromatographic methods, Rice, Speciation.

## INTRODUCTION

Mercury (Hg) pollution is considered a major environmental and public health concern. Because of its toxicity, Hg has been recently included in the top ten hazardous chemicals by the World Health Organization (WHO). Pregnant women and children in early life are considered the most vulnerable population to Hg harmfulness. Toxic effects can be lethal and include infections of the nervous, digestive and immune systems, and lungs and kidneys.

* **Corresponding author Zoyne Pedrero Zayas:** CNRS/UNIV PAU & PAYS ADOUR, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux, UMR 5254, 64000, Pau, France; Tel: +33 (0)5 40 17 5027; E-mail: zoyne.pedrerozayas@univ-pau.fr

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

Taking into account the capability of Hg to bind thiols [1], its interaction with essential proteins and enzymes leading to their dysfunction seems to be the origin of such toxicity.

Despite occupational exposure (*i.e.*, miners, dentists) - dental amalgams being undisputable sources of Hg - diet appears as the main exposure pathway of Hg in humans. In general, seafood consumption is recognized as the most common pathway of Hg human exposure. It is especially troubling considering the recent and significant increase of Hg in oceanic waters [2]. Anthropogenic activities, such as mining and coal burning are responsible for the increased Hg levels in the atmosphere and in oceanic surfaces [3]. Microorganisms in aquatic ecosystems play a crucial role since they biotransform inorganic Hg (iHg) into methylmercury (MeHg which is present in its free form as $CH_3Hg^+$). The latter exhibits high levels of toxicity and it is easily bioaccumulated through the food chain resulting in serious social and health effects.

Considering Hg toxicity, food safety authorities set the maximal acceptable levels for Hg in food. The established Hg values in foodstuffs depend on their nature. For food supplements, the maximum level is as high as 0.10 mg $kg^{-1}$ in the final product. In the case of fishery products comprising crustaceans and muscle meat of fish (except predatory ones), it is fixed at 0.5 mg $kg^{-1}$ wet weight and for predatory fish species as bonito, eel, marlin, sharks and tuna, among others, it is 1 mg $kg^{-1}$ wet weight (COMMISSION REGULATION (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs). These regulations are effective for fresh and processed fish.

A tolerable weekly intake (TWI) has been set as a safe consumption threshold in order to avoid Hg exposure risks and is regularly reevaluated. The European Food Safety Authority set in 2012 a new MeHg TWI at 1.3 μg $kg^{-1}$ bodyweight, lower than that established by the Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives of 1.6 μg $kg^{-1}$ bodyweight. However, due to seafood consumption, a significant part of the global population, mainly from developing countries, is exposed to higher Hg levels than the thresholds established by food safety authorities [3].

Taking into account that diet is the principal source of exposure to Hg, its toxicity is strongly related to its speciation, and Hg species are toxic at low concentrations, the analytical chemistry community is continuously seeking for advances in foodstuffs analysis methods. Currently, the main goals of the new and trendy analytical approaches are the development of sensitive, cost-effective and green methods for the determination of Hg and its species. Isotopic fractionation analysis also appears as a fresh strategy for the identification and discrimination

of Hg sources in food products, adding another dimension to total Hg quantification and speciation. In this chapter, current and promising approaches for Hg analysis in food are described.

## FOOD MATRICES WHERE HG IS OFTEN DETERMINED

According to a recent report of FAO (2016), fisheries and aquaculture are very important sources of food, nutrition, income and livelihood for hundreds of millions of people around the world. Driven by rising domestic income, consumers in emerging economies (where consumption was previously based on locally available products) are experiencing a diversification of the types of available fish through an increase in fishery imports. The significant growth in fish consumption has enhanced people's diets around the world through diversified and nutritious food. Fish consumption represents in many countries the dominant source of proteins. Therefore, the high seafood consumption could lead to significant risks due to MeHg ingestion.

Since seafood is considered a major contributor of Hg through diet, the quantification of Hg and its species in such products has grabbed the attention of the analytical chemistry community. As a consequence, most of the Hg speciation studies in foodstuffs correspond to the analysis of fish and other seafood. The new methodologies developed for Hg speciation in fish and seafood are presented all along the text.

Rice is a dominant global crop, recognized to be one of the most important sources of Hg in human diet. Microbial Hg methylation is considered the main source of this organomercurial species in paddy soils. The traditional rice culture practice involves several flooding processes, which lead to anaerobic conditions facilitating iHg methylation by sulfate reducing bacteria [4]. In addition, the use of iodomethane as fumigant, enhances Hg methylation in soil under sunlight, increasing MeHg exposure from rice [5]. After soil uptake by the plant, Hg is transported to the edible part [6, 7]. It constitutes a potential risk in Hg polluted areas like Hg-contaminated mining regions, where Hg values reach up to 500 ng $g^{-1}$ [7]. In such regions, the most important MeHg exposure source is not fish, but rice consumption [8]. MeHg intake through rice ingestion has been reflected on the levels of MeHg in hair of inhabitants of such areas [7, 9].

Rice seeds consist of a hull and a nutritious bran coat layer surrounding the endosperm and inner embryo. Brown rice is the result of removing the hull (inedible) and can be consumed in this state. Further processing yields to "white" or "polished" rice. The distribution of Hg species varies according to the fraction of the rain grains. Mostly, iHg is located in hull and bran, while MeHg is found in edible white rice. Rice processing leads to a release of up to 78% of iHg, which is

**CHAPTER 9**

# Process Contaminants

**Marta Mesías, Francisca Holgado** and **Francisco J. Morales***

*Institute of Food Science, Technology and Nutrition, Spanish National Research Council (ICTAN-CSIC), C/ José Antonio Novais, 10. 28040 Madrid, Spain*

**Abstract:** Contaminants are substances that may be present in foods as a result of production, preparation, food formulation, processing, packaging, transport and storage, as well as a result of environmental contaminant. Among them, process contaminants are generated in foods due to chemical reactions occurring during cooking, processing and preservation and are considered to exert adverse toxicological effects in humans. This chapter focuses on some of these process contaminants, specifically on contaminants formed after thermal treatment of foods, such as acrylamide, furan, heterocyclic aromatic amines, chloropropanodiols and their esters, glycidol and glycidyl esters. Heat-generated food contaminants are mostly produced during cooking at high temperatures as a result of Maillard reaction and lipid oxidation, although other non-thermal reactions may also contribute to their formation. Characterization, toxicological considerations, chemical formation, occurrence and exposure are detailed, as well as mitigation strategies applied to prevent their formation and/or reduce and remove from the processed food.

**Keywords:** Acrylamide, Analysis, Chemical reaction, Cooking, Diet, Exposure, Food safety, Furan, Glycidol esters, Heat, Heterocyclic aromatic amines, Intake, Maillard reaction, Mitigation, 3-monochloropropanediol, Preventive strategies, Process contaminants, Risk, Toxic, Xenobiotics.

## INTRODUCTION

### General Considerations to Food Safety

Food safety is an activity related to the evaluation of microbiological, chemical and physical hazards that cover handling, preparing and storing food in ways that prevent food-borne illness. In the past, major attention on food safety has been given to food microbiology issues resulting from unexpected and sudden microorganism contamination and outbreak. Since mid of the last century, food

* **Corresponding author Francisco J. Morales:** Institute of Food Science, Technology and Nutrition, Spanish National Research Council (ICTAN-CSIC), C/ José Antonio Novais, 10. 28040 Madrid, Spain; Tel: +34 915492300; E-mail: fjmorales@ictan.csic.es

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

hygiene does not only address terms of microbiological health hazards,but also chemical food contamination. Chemicals are essentially present in practically every place in the world, including living organisms, and of course in all foods. In foods, these are predominantly proteins, saccharides, lipids, low-molecular organic compounds, minerals and water. The nature of the chemical substances confers their toxicological properties. However, most of the chemicals present in our foods are harmless contributing to the nutritional, technological and sensorial properties of foods, but also being necessary to participate into the physiological reactions in the living organism. In that sense, food contains (naturally, intentionally or additives) a wide range of substances. Among the intentionally added additives, the regulated additives are largely known. Additives are important to increase the nutritional value of foods (vitamins, minerals, amino acids), the sensory properties (pigments, sweeteners, flavoring and its enhancers), and the shelf life of foods (antimicrobials, antioxidants).

Xenobiotics can be described as any foreign chemical in the food that may endanger human safety since they are biochemically active substances. Once they enter into the organism, they can induce or inhibit metabolic pathways, which could be related to enzymes, or transporters expressed in the human host, as well as the microbiota of the gastrointestinal tract. Xenobiotics can be classified into broad categories, according to their relevance in terms of food safety, namely contaminants and chemicals that have been intentionally added to food or raw commodities [1]. Some examples are those potentially present in raw foods (residues of veterinary drugs, environmental pollutants, fertilizers), those intentionally introduced during technological processing, chemicals passed from the packaging and processing equipment, is formed during storage.

The presence of xenobiotics in foods is practically unavoidable. However, the application of food security systems in EU, such as hazard analysis and critical control points (HACCP) and good hygienic practice (GHP), together with a continuous updated legislation, is a guarantee to avoid or even reduce to an acceptable level, the occurrence of most of the harmful xenobiotics to foods [2]. The *Codex Alimentarius*, established in 1963 under FAO/World Health Organization Commission, provides a basis for regulations in order to control the content of hazardous chemicals in foods. Food safety should be a guarantee that no adverse effects occur in humans after food ingestion, ultimately having an impact on human health and wellness.

## Risk Assessment Scheme

Hazard control in foods is supervised by supranational official authorities, governmental agencies and the scientific community. There are a number of food

safety regulatory bodies and international organizations which are responsible for the food safety, such as *Codex Alimentarius* Commission, World Health Organization (WHO), Food and Agricultural Organization (FAO), World Trade Organization, US Food and Drug Administration (FDA), Food Standards Australia New Zealand, among others. In Europe, the European Food Safety Authority (EFSA) is the keystone of European Union risk assessment regarding food and feed safety that works in close collaboration with national authorities. EFSA, established in 2002, provides independent scientific advice and clear communication on existing and emerging risks. The remit of EFSA concerns the entire food chain covering aspects of human, animal and plant health, and, sometimes, environmental protection. Its focus in human health is on food safety and its scientific advice may contribute to various phases in the policy cycle: reflection, regulation, verification and review. The EU follows the as-low-as reasonably-achievable (ALARA) principle of decision-making for any risk assessment advice. The Scientific Panel of Food Chain Contaminants (CONTAM) would assist EFSA's scientific advice for chemical contaminants.

It is important to describe the differences between hazard and risk as defined by the General Food Law in Europe [3]. Hazard is defined as - a biological, chemical or physical agent in, or condition of, food or feed with the potential to cause an adverse health effect -, while 'risk' means - a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard.

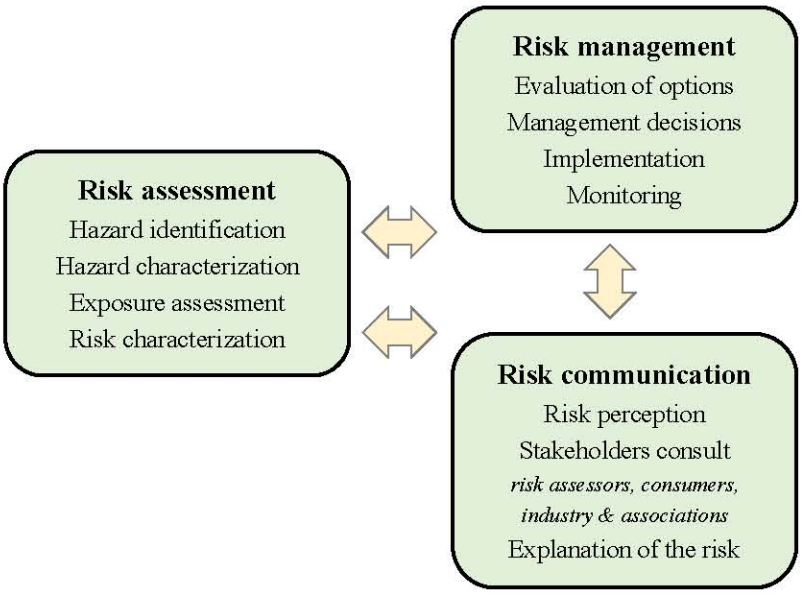


**Fig. (1).** Risk analysis framework.

CHAPTER 10

# Mycotoxins

**Yelko Rodríguez-Carrasco**[1,*] and **Alberto Ritieni**[2,*]

[1] *University of Valencia, Department of Food Chemistry and Toxicology, Av/ Vicent A. Estellés, s/n 46100 Burjassot, Valencia, Spain*

[2] *Università di Napoli Federico II, Department of Pharmacy, Via D. Montesano, 49 - 80131Napoli, Italy*

**Abstract:** Mycotoxins are secondary metabolites produced by fungal species which can usually be found in foodstuffs. The effects of some food-borne mycotoxins are acute, symptoms of severe illness appearing very quickly. Other mycotoxins occurring in food have longer term chronic or cumulative effects on health, including the induction of cancers and immune deficiency. Thus, Regulation (EC) 1881/2006, partially amended by other Regulations, set maximum contents of some mycotoxins in different foodstuffs allowing to evaluate risks and take actions to protect public health. In this chapter, mycotoxins with significant health and food production impact are discussed by considering the following items: chemical structure, conditions of their production, occurrence in food, maximum limits, toxicity and analytical methods. The chapter also includes the exposure assessment approach to these food contaminants, their metabolism and the proposed biomarkers in the literature. A final remark about the toxicogenomic approach is also included in the chapter as a future trend in the study of mycotoxins.

**Keywords:** Aflatoxins, *Alternaria* toxins, Biomarkers, Chromatography, Emerging fusariotoxins, Exposure assessment, Food, Fumonisins, Mass spectrometry, Metabolism, Metabolites, Mycotoxins, Ochratoxin A, Occurrence, Patulin, Trichothecenes, Toxicity, Zearalenone.

## GENERAL INTRODUCTION

### Preamble

The term mycotoxin derived from Greek words mikes (fungus) and toxicum (poison).

[*] **Corresponding author Yelko Rodríguez Carrasco and Alberto Ritieni:** University of Valencia, Department of Food Chemistry and Toxicology, Av/ Vicent A. Estellés, s/n - 46100 Burjassot, Valencia, Spain; Tel: +34 963543047; Fax: +34 963544284; E-mail: yelko.rodriguez@uv.es and Università di Napoli Federico II, Department of Pharmacy, Via D. Montesano, 49 - 80131 Napoli, Italy; Tel: +39 081678652; Fax: +39 081678610; E-mail: alberto.ritieni@unina.it, respectively

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

Mycotoxins are defined as secondary fungal metabolites, low molecular weight (MW <800 Da), produced by filamentous fungi whose ingestion, inhalation or dermal absorption can cause various diseases and disorders known as mycotoxicosis, the severity of which depends on the toxicity of mycotoxin, the degree of exposure, age and nutritional status of both human and animal [1, 2].

The oldest mycotoxicosis described in human is the Ergotism, a condition described in the Middle Age and caused by the consumption of plant products contaminated by ergot alkaloids produced by the fungus *Claviceps purpurea* which triggered several epidemics that devastated Western Europe [3]. The most important case of human mycotoxicosis by trichothecenes was described as endemic in parts of Russia in 1932 causing high mortality rates (60% of those affected). Initially it was thought that the disease had an infectious origin and could even be due to a vitamin deficiency so confused with diseases such as scarlet fever, diphtheria, pellagra and even scurvy, but was finally in 1943 when it was named as "leukopenia toxic hemorrhagic", better known as ATA (*Alimentary toxic aleukia*) caused by contamination of crops by T-2 toxin; a toxin produced by *Fusarium sporotrichoides* [4]. The discovery of aflatoxins in the 60s of the 20$^{th}$ century marked a turning point in the study of Mycotoxicology, when thousands of young turkey and other birds died in the England because of an illness which was coined as "turkey disease X" due to the consumption of peanut flour contaminated by toxins from *Aspergillus flavus* [3, 4].

Since then, there have been significant amount of research conducted to determine the presence and toxicity of mycotoxins in various food matrices and which have driven the development of strategies for detoxification of these toxic compounds to ensure food safety [5].

## OVERVIEW

Among the major mycotoxin producers molds are included those within the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* or *Claviceps*. Those fungi under certain conditions (temperature, humidity, water activity, pH, substrate composition, etc.) can colonize and subsequently contaminate with mycotoxins food and feed (Table **1**) [1].

These fungi are widely distributed worldwide. *Aspergillus* genera is usually isolated in tropical area, whereas *Fusarium* and *Penicillium* are mainly found in cold climates and in temperate zones, respectively. Nonetheless, there is not a clear pattern at this moment due to the climate change [6, 7]. Fig. (**1**) shows the global map of mycotoxin occurrence and risk in different regions.

**Table 1. Conditions of temperature and water activity (aw) for the growth of the main mycotoxigenic fungi and the production of their toxins.**

| Species | Growth | | Toxin Production | | |
|---|---|---|---|---|---|
| | Temperature | Minimum aw | Mycotoxin | Minimum aw | Optimal aw |
| *Aspergillus flavus* | 24 °C to 37 °C | 0.78 | AFs | 0.80-0.82 | 0.95-0.98 |
| *Aspergillus parasiticus* | | 0.80 | | 0.83 | 0.98 |
| *Aspergillus ochraceus* | 24 °C to 37 °C | 0.77 | OTA | 0.80-0.90 | 0.95 to 0.99 |
| *Penicillium verrucosum* | 20 °C to 32 °C | 0.80 | | 0.83-0.85 | 0.90-0.99 |
| *Penicillium expansum* | 23 °C to 27 °C | 0.83 | PAT | - | 0.98 |
| *Fusarium verticillioides* | 25 °C to 37 °C | 0.90 | FBs | - | 0.97 |
| *Fusarium proliferatum* | | 0.90 | | - | 0.97 |
| *Fusarium sporotrichioides* | 15 °C to 27 °C | 0.90 | ZON and TCs | 0.95 | 0.97 to 0.99 |
| *Fusarium graminearum* | | 0.90 | | 0.95 | 0.97 to 0.99 |
| *Fusarium culmorum* | | 0.90 | | 0.95 | 0.97 to 0.99 |

AFs: aflatoxins; OTA: ochratoxin A; PAT: patulin; FBs: fumonisins; ZON: zearalenone; TCs: trichothecenes

The invasion by these fungi may occur during the pre-harvest (field) or post-harvest stages (storage, transport and processing), causing both serious economic losses and health problems among humans and livestock [8]. It has to be highlighted that a same toxigenic strain can produce various mycotoxins, and one mycotoxin can be synthesized by different fungi. These metabolites have different chemical structures and biological activities. According to the literature, there have been described about 400 mycotoxins, being the most important due to their adverse health effects on human and animals the followings: aflatoxins, ochratoxin A, fumonisins, trichothecenes, zearalenone and patulin [9].

The presence of mycotoxins along the food chain remains a major public health problem [10 - 12]. In this sense, the Food and Agriculture Organization of the World Health Organization (FAO/WHO) has estimated that at least 25% of the world's crops are contaminated with mycotoxins [13]. Table **2** shows the most frequent combination of contaminated food and type of mycotoxin. In this line, mycotoxins are within the food contaminants with the highest number of notifications according to the annual reports published by the Rapid Alert System for Food and Feed (RASFF) [14]. This trend has been maintained over time adjusting to the natural fluctuations of these contaminants. Table **3** shows the total notifications of mycotoxins since 2004. Table **4** shows a comparison of the number of notifications recorded in recent years for mycotoxins, pathogenic microorganisms and pesticide residues.

CHAPTER 11

# Biogenic Amines

**Gianni Sagratini***, **Giovanni Caprioli, Massimo Ricciutelli** and **Sauro Vittori**

*University of Camerino, School of Pharmacy, Via S. Agostino 1, 62032 Camerino (MC), Italy*

**Abstract:** Biogenic amines (BAs) are basic molecules present in food formed by decarboxylation of aminoacids of proteins. They have a particular profile from a toxicological point of view, and the intake of food with high presence of BAs can generate various problems and allergic responses. Due to the importance of their toxicological aspects, BAs are considered as an important indicator of freshness and quality of food, through the evaluation of specific indices that take into account their concentration in food, *i.e.*, Biogenic Amine Index (BAI) or the ratio spermidine/spermine (SPD/SPM). Many foods can be contaminated by the high levels of BAs as meat, cheese, fish, beer, wine and baby foods, and no regulation exists by EFSA or FDA except for histamine in fish. The analytical methodologies used for the detection of the BAs in food are normally based on a primary step of sample preparation (extraction and purification) and then on a second step of instrumental analysis that uses high performance liquid chromatography (HPLC) or gas chromatography (GC) coupled to various detectors as diode array detector (DAD), fluorescence detector (FD), mass spectrometry (MS) and tandem mass spectrometry (MS/MS). Also capillary electrophoresis (CE) has been used for the analysis of BAs in food. This chapter describes an overview on the presence of BAs in foods and the most important analytical strategies for their analysis and detection.

**Keywords:** Biogenic amines, CE, DAD, Derivatization, FD, GC, HPLC, MS proteic food, Sample preparation, Shelf life markers.

## INTRODUCTION

Biogenic amines (BAs) are basic molecules of low molecular mass present in living organisms and, hence, in food [1]. Based on their chemical structures, they are classified into three categories: (1) aromatic, as histamine, tryptamine, tyramine, 2-phenylethylamine, (2) aliphatic diamines, as cadaverine and putrescine, and (3) aliphatic polyamines, as spermine and spermidine (Fig. **1**) [2].

BAs are biologically active molecules that are involved in many cellular functions; monoamines play an important role in neurotransmission and the reg-

* **Corresponding author Gianni Sagratini:** University of Camerino, School of Pharmacy, Via S. Agostino 1, 62032 Camerino (MC), Italy; Tel: +39 0737 402238; E-mail: gianni.sagratini@unicam.it

**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

ulation of blood pressure, polyamines are essential for cellular proliferation and differentiation as they participate in the synthesis of DNA, RNA, and proteins [3].

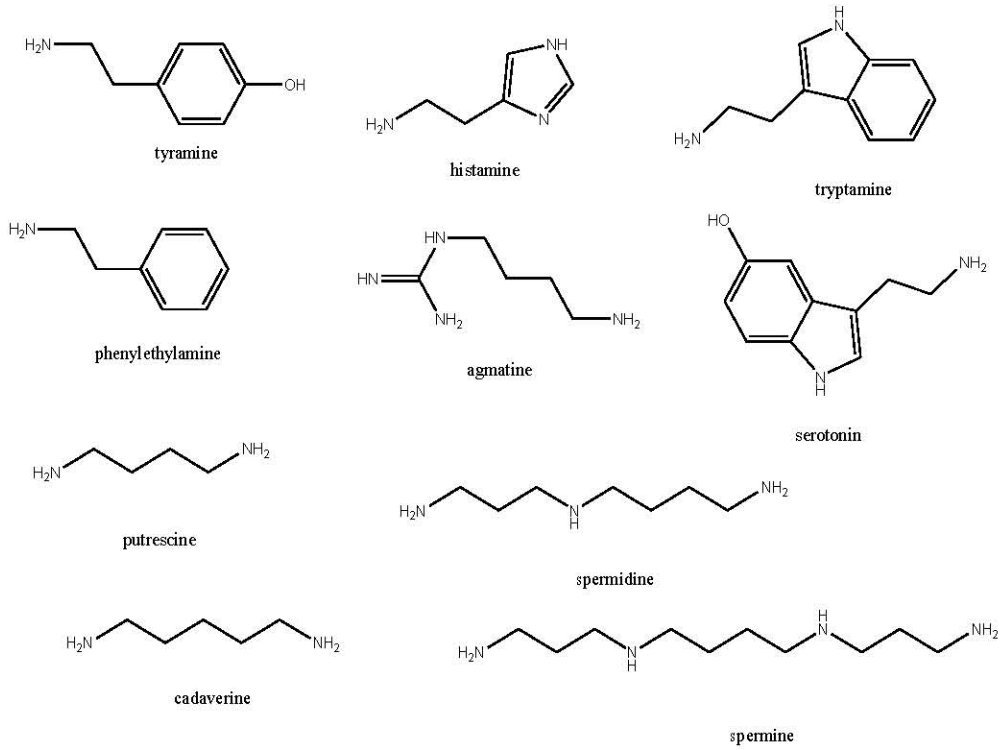


**Fig. (1).** Structures of the most relevant biogenic amines occurring in food.

BAs are produced in food of proteic origin by three possible mechanisms: (a) decarboxylation of aminoacids (promoted by the decarboxylase enzymes present in various microorganisms) [4], normal cellular metabolism of tissues [5], amination or transamination of aldehydes and ketones [4].

BAs are important from a toxicological point of view, because the intake of food with high concentrations of BAs can generate migraine, headaches, gastrointestinal disorders, and allergic responses. Histamine poisoning produces effects on cardiovascular system such as low blood pressure [6], while tyramine causes allergic skin reactions and increasing blood pressure by releasing noradrenaline from the sympathetic nervous [5]. Other amines, such as spermidine or spermine, have also been associated with the development of food allergy. In normal conditions, the human body can detoxify histamine and tyramine coming from foods by acetylation and oxidation mediated by the

enzymes monoamine oxidase (MAO), diamine oxidase (DAO), and polyamine oxidase (PAO) [5]. However, if these detoxifying mechanisms are upset because there is a lack of aminooxidases, BAs increased their concentration in the body and could cause serious toxicological problems. Putrescine and cadaverine, although not considered toxic individually, can increase the effect of histamine and tyramine by interacting with aminooxidases and decreasing with the detoxifying mechanism [7]. It is really very difficult to establish limits of toxicity of BAs in food, because their effect does not depend on their presence alone but is also influenced by other molecules and by the ability of the detoxifying mechanisms.

Literature reports that BAs are potential precursors for the formation of carcinogenic N-nitroso compounds. The reaction of primary amines and nitrosating-agents produces alkylating species, which can react with other food components by generating toxic compounds [8]. The secondary amines such as agmatine, spermine, spermidine and others can react with nitrile and produce the nitrosamines, while tertiary amines produce a range of labile N-nitroso derivatives [9].

Due to the importance of their toxicological aspects, BAs are considered as an important indicator of freshness and quality of food [10]. Various indices can be used for evaluating the quality of fresh food, firstly Biogenic Amine Index (BAI) that is the sum (mg $kg^{-1}$) of putrescine, cadaverine, histamine and tyramine, then the ratio between spermidine and spermine (SPD/SPM) and the total sum of analyzed BAs. Also the Chemical Quality Index (CQI) has been taken into account for evaluating the quality of food, in particular fish; it is calculated by the sum of the concentration of putrescine + cadaverine + histamine divided by spermine + spermidine + 1. In particular, a CQI between 0 and 1 indicates good quality tuna, between 1 and 10, borderline, and, higher than 10, decomposed [11].

Although BAs have been described as potential toxic compounds, the maximum histamine level is only regulated in fishery products, at 50 mg $kg^{-1}$ by the US Food and Drug Administration (FDA), and at 100 mg $kg^{-1}$ by the European Community [12]. However, the European Food Safety Authority (EFSA) produce a scientific opinion where it describes the risks related to the intake of BAs in fermented products [13]. This document highlights the importance of controlling these molecules in food, and the need to validate analytical methods for their detection. On the other hand, some European countries recommended fixed limits for histamine in wine [Germany (2 mg $L^{-1}$), Belgium (5-6 mg $L^{-1}$), and France (8 mg $L^{-1}$)] [14, 15].

Food safety has promoted more research in the field of BAs in the last few years,

# SUBJECT INDEX

## A



## B



**Belén Gómara & María Luisa Marina (Eds.)**
**Current and Future Developments in Food Science (Vol. 1)**

## C



## D

## E



## F

## G

## H



## I

## J



## L



## M

## N



## O



## P

## Q



## R



## S

## T



## U



## V



## W



## Y



## Z

## Belen Gomara

Belen Gomara is a Tenured Scientist at the Department of Instrumental Analysis and Environmental Chemistry of the Institute of Organic Chemistry (IQOG) of the Spanish National Research Council (CSIC) in Madrid (Spain) from 2008. Her research career has been enclosed in the areas of Analytical Chemistry, Environmental Chemistry and Food Safety. She focused on the development and validation of analytical methodologies for the determination of legislated and emerging-non-legislated persistent organic pollutants (POPs). In addition, her current scientific activity involves the development of methodologies for the determination of contaminants related to food packaging, such as phthalates and bisphenols (including bisphenol A (BPA) and its substitutes), as well as, to study the presence of these compounds in foodstuffs commercially available and to carry out studies on the migration of plasticizers from food contact materials.

## Maria Luisa Marina

Maria Luisa Marina is Full Professor of Analytical Chemistry at the Faculty of Sciences of the University of Alcalá (Madrid, Spain) from 2004. Her research activity is focused on the development of innovative analytical methodologies using capillary chromatographic and electrophoretic techniques for the determination of compounds of interest in the pharmaceutical, environmental and food analysis fields including the enantiomeric separation of chiral compounds, the search for biomarkers using metabolomics strategies and the determination of proteins, peptides and amino acids with important applications to the control of the quality and safety of foods and the revalorization of food residues. Prof. Marina has supervised 20 PhD Theses and headed or participated in numerous research projects. She is the co-editor of the book "Analysis and detection in capillary electrophoresis", Comprehensive Analytical Chemistry Series (Elsevier) and a co-author of numerous international book chapters and more than two hundred research articles (SCI).