

# Contents

Preface — V

Contributors — XIII

Natalia Vilariño

- 1 High-throughput detection methods — 1**
- 1.1 Introduction — 1
- 1.2 Laboratory automation — 1
- 1.3 Miniaturization and parallelization of detection technologies — 3
- 1.4 Assay design — 7
- 1.4.1 Immunodetection — 7
- 1.4.2 Receptor-based methods — 10
- 1.4.3 Enzymatic activity — 11
- 1.4.4 Aptamer-based detection — 11
- 1.4.5 Cell-based assays — 12
- Acknowledgments — 14
- References — 14

Inés Rodríguez and Amparo Alfonso

- 2 Analytical instrumentation and principles — 17**
- 2.1 Introduction — 17
- 2.2 Sample/matrix extraction — 20
- 2.2.1 Sample preparation — 20
- 2.2.2 Extraction procedure — 20
- 2.2.2.1 Extraction procedures from solid matrices (solid–liquid extraction) — 20
- 2.2.2.2 Extraction procedures from liquid matrices (liquid–liquid extraction) — 21
- 2.3 Chromatography — 21
- 2.3.1 Classification — 22
- 2.3.1.1 Based on phase combination — 22
- 2.3.1.2 Based on the mechanism of separation — 22
- 2.3.1.3 Based on phase polarity — 22
- 2.3.1.4 Based on the shape of chromatography bed — 23
- 2.3.1.5 Based on the development procedure — 23
- 2.4 Column chromatography – Solid phase extraction (SPE) and sample preparation — 23
- 2.5 High-Performance Liquid Chromatography (HPLC) analysis — 26
- 2.5.1 Components of liquid chromatograph — 27
- 2.5.1.1 Mobile phases and elution — 27
- 2.5.1.2 Pumps — 28

- 2.5.1.3 Injection — 28
- 2.5.1.4 Columns — 29
- 2.5.1.5 Oven — 29
- 2.5.1.6 Detectors — 31
- 2.6 Liquid chromatography for toxin identification — 39
- 2.6.1 Lipophilic toxins — 39
- 2.6.2 Hydrosoluble marine toxins: *PSPs*, *TTXs*, *DA* — 40
- 2.6.3 Cyanotoxins — 46
- 2.6.4 Mycotoxins — 46
- 2.6.5 Ciguatoxins — 51
- 2.6.6 Identification of unknown and novel toxins — 51
- References — 55

Carmen Alfonso and Álvaro Antelo

- 3 Quantitative and qualitative methods, primary methods — 58**
- 3.1 Quantitative methods: Beyond the numerical data — 58
- 3.2 Metrological traceability — 64
- 3.3 Method of analysis: Primary methods — 67
- 3.4 Qualitative methods: A new focus on analytical chemistry — 72
- 3.5 Reference materials: Characteristics, applications, and producers — 79
- References — 88

M. Carmen Louzao and Paula Abal

- 4 Toxicological studies with animals — 91**
- 4.1 History of toxicity studies with animals and legislation — 91
- 4.1.1 The three Rs — 92
- 4.2 Experimental animals — 93
- 4.3 Administration routes — 96
- 4.4 Types of toxicity studies with animals — 99
- 4.4.1 Acute toxicity testing — 101
- 4.4.2 Repeated dose toxicity testing — 105
- 4.4.3 Subchronic toxicity testing — 108
- 4.4.4 Chronic toxicity testing — 109
- 4.4.5 Toxicokinetic studies — 110
- 4.5 Future of toxicity studies with animals — 112
- Acknowledgments — 112
- References — 113

Carmen Vale González, Andrea Boente Juncal and Aida González Méndez

- 5 Toxicological studies with cells — 115**
- 5.1 Introduction — 115

- 5.2 *In vitro* culture conditions — 115
- 5.3 Handling and maintenance of cell cultures — 117
- 5.4 *In vitro* culture models — 119
- 5.5 *In vitro* cytotoxicity tests and methods to evaluate cellular function — 123
- 5.6 *In vitro* methods to evaluate the effects of compounds on reproduction — 125
- 5.7 *In vitro* methods in cancer research — 126
- 5.8 *In vitro* methods for environmental toxicity — 126
- 5.9 *In vitro* methods for basal toxicity and cytotoxicity — 128
- 5.10 *In vitro* methods to test cellular function — 140
- Acknowledgments — 142
- References — 143

Eva Alonso and Rebeca Alvaríño

- 6 Marine Toxins — 146**
- 6.1 Introduction — 146
- 6.2 Lipophilic toxins — 151
  - 6.2.1 Pectenotoxins — 151
  - 6.2.2 Yessotoxins — 154
  - 6.2.3 Azaspiracid — 155
  - 6.2.4 Okadaic acid and dinophysistoxins — 155
  - 6.2.5 Ciguatoxins — 156
  - 6.2.6 Brevetoxins — 156
  - 6.2.7 Gambierol — 157
  - 6.2.8 Cyclic Imine toxins — 157
- 6.3 Hydrophilic toxins — 158
  - 6.3.1 Saxitoxin and analogs — 158
  - 6.3.2 Domoic acid — 160
  - 6.3.3 Tetrodotoxin — 160
- 6.4 Amphiphilic toxins — 161
  - 6.4.1 Maitotoxin — 161
  - 6.4.2 Palytoxin — 162
- Acknowledgments — 163
- References — 163

Eva Cagide and Mercedes Álvarez

- 7 Cyanobacterial toxins — 168**
- 7.1 Cyanobacteria and algal blooms — 168
- 7.2 Cyanotoxins — 170
  - 7.2.1 Microcystins — 173
    - 7.2.1.1 Mode of action — 173

7.2.1.2	Chemical structure —	<b>174</b>
7.2.2	Nodularins —	<b>179</b>
7.2.2.1	Mode of action —	<b>179</b>
7.2.2.2	Chemical structure —	<b>180</b>
7.2.3	Neurotoxic alkaloids: anatoxins and saxitoxins —	<b>181</b>
7.2.4	Anatoxin-a and homoanatoxin-a —	<b>182</b>
7.2.4.1	Mode of action —	<b>182</b>
7.2.4.2	Chemical structure —	<b>182</b>
7.2.5	Anatoxin-a(S) —	<b>183</b>
7.2.5.1	Mode of action —	<b>183</b>
7.2.5.2	Chemical structure —	<b>184</b>
7.2.6	Saxitoxins —	<b>184</b>
7.2.6.1	Mode of action —	<b>184</b>
7.2.6.2	Chemical structure —	<b>185</b>
7.2.7	Cylindrospermopsin —	<b>187</b>
7.2.7.1	Mode of action —	<b>187</b>
7.2.7.2	Chemical structure —	<b>187</b>
7.2.8	Aplysiatoxins and lyngbyatoxins —	<b>189</b>
7.2.8.1	Mode of action —	<b>189</b>
7.2.8.2	Chemical structure —	<b>189</b>
7.2.9	Lipopolysaccharides —	<b>190</b>
7.2.9.1	Mode of action —	<b>190</b>
7.2.9.2	Chemical structure —	<b>190</b>
7.2.10	BMAA —	<b>190</b>
7.2.10.1	Mode of action —	<b>190</b>
7.2.10.2	Chemical structure —	<b>191</b>
7.3	Detection methods —	<b>191</b>
7.4	Health aspects: Guidelines and legislation —	<b>194</b>
	References —	<b>196</b>

María J. Sainz, Jesús M. González-Jartín, Olga Aguín, J. Pedro Mansilla and Luis M. Botana

<b>8</b>	<b>Isolation, characterization, and identification of mycotoxin-producing fungi —</b>	<b>202</b>
8.1	Introduction —	<b>202</b>
8.2	Major mycotoxins and their toxicity —	<b>204</b>
8.2.1	Aflatoxins —	<b>207</b>
8.2.2	Ochratoxins —	<b>208</b>
8.2.3	Trichothecenes —	<b>208</b>
8.2.4	Zearalenone —	<b>209</b>
8.2.5	Fumonisin —	<b>210</b>
8.2.6	Patulin —	<b>210</b>

8.3	Main mycotoxin-producing fungi — 211
8.3.1	Fungi: An overview — 212
8.3.2	Life cycle of Ascomycota — 213
8.3.2.1	Sexual reproduction — 214
8.3.2.2	Asexual reproduction — 215
8.3.3	Naming of filamentous Ascomycota — 215
8.3.4	<i>Fusarium</i> — 216
8.3.4.1	Morphological characteristics of <i>Fusarium</i> species — 216
8.3.5	<i>Aspergillus</i> and <i>Penicillium</i> — 219
8.3.5.1	<i>Aspergillus</i> — 219
8.3.5.2	Main morphological characteristics of <i>Aspergillus</i> species — 220
8.3.5.3	<i>Penicillium</i> — 222
8.3.5.4	Main morphological characteristics of <i>Penicillium</i> species — 222
8.4	Methods for isolation, identification, and characterization of mycotoxigenic fungi — 224
8.4.1	Detection and isolation of mycotoxigenic fungi — 225
8.4.1.1	Media for isolation of <i>Fusarium</i> , <i>Aspergillus</i> , and <i>Penicillium</i> species — 227
8.4.1.2	Plating methods — 227
8.4.2	Obtaining monosporic fungal isolates — 230
8.4.3	Morphological characterization — 230
8.4.4	Molecular and phylogenetic identification — 234
8.4.4.1	Extraction of genomic DNA — 236
8.4.4.2	PCR amplification and sequencing — 237
	Acknowledgments — 239
	References — 240
Ana M. Botana	
9	<b>Analysis of environmental toxicants — 246</b>
9.1	Introduction — 246
9.2	General guidelines of chemical analysis in the environment — 247
9.2.1	Isolation (extraction and separation) — 248
9.2.2	Separation and purification — 248
9.2.3	Sample concentration — 249
9.2.4	Measurement: Instrumental analytical methods — 251
9.3	Atomic spectrometry — 251
9.4	Gas chromatography — 252
9.5	Liquid chromatography (LC) — 256
	References — 261