Contents

List of Contributors XIX Preface XXV A Personal Foreword XXVII

Part I

1	Process Logistics, Testing Strategies and Automation Aspects 3
	Hansjoerg Haas, Robert S. DeWitte, Robert Dunn-Dufault,
	and Andreas Stelzer
1.1	Introduction 3
1.2	The Process from Raw Ingredients to Data 3
1.2.1	Compound Management 5
1.2.2	Cell Biology 6
1.2.3	Lead Profiling 7
1.2.4	Liquid Chromatography/Mass Spectrometry 7
1.3	DMPK Testing Strategies: the Process from Data to Decisions 8
1.4	New Questions, New Assays and New Technologies Challenge
	the Process 10
1.5	Organizational Models to Scale Up the Process 11
1.5.1	Food Court 11
1.5.1.1	The Fast Food Restaurant 12
1.5.1.2	The Family Restaurant Chain 12
1.6	Critical Factors to Improve the Process 13
1.7	Materials in ADME/Tox Screening 14
1.8	Machines and Equipment in ADME/Tox Screening 17
1.8.1	Liquid Handlers 17
1.8.2	Detection and Analysis 17
1.9	Software, Data Retrieval, Analysis, Manipulation and Interpretation 18
1.10	Environment and Management = Organizational Structure in
	ADME/Tox Screening 19

VI	Contents	
	1.11 1.11.1 1.11.1.1 1.11.1.2 1.11.1.3 1.12	Methods in ADME/Tox Screening 20 Examples of Whole-Process Approaches 20 Automation Islands with Manual Data Upload to a LIMS System 21 Complete Physical Integration and Automation 21 Federated Physical Automation with Software Integration 22 Conclusions 22 References 23
	2	Prediction of Drug-Likeness and its Integration into the Drug Discovery Process 25 Ansgar Schuffenhauer and Meir Glick
	2.1	Introduction 25
	2.2	Computational Prediction of Drug-Likeness 26
	2.2.1	Machine Learning 26
	2.2.2	Empirical Rules and Their Basis 30
	2.2.3	Drug-Likeness of Natural Products 32
	2.2.4	Do Ligands of Different Target Classes Differ in Their Drug-Like
		Properties? 34
	2.2.5	Unwanted Structural Elements 34
	2.3	What is the Best Practice in Utilizing Drug-Likeness in Drug
	2.4	Discovery? 35 Concluding Discussions 37 References 38
	3	Integrative Risk Assessment 41 Bernard Faller and Laszlo Urban
	3.1	The Target Compound Profile 41
	3.1.1	Introduction 41
	3.1.2	The Importance of the Projected Clinical Compound Profile in Early Drug Discovery 42
	3.1.3	The Impact of Delivery On the Design of the Drug Discovery Process 43
	3.2	The Concept of Hierarchical Testing in Primary and Follow-Up Assays 45
	3.2.1	Impact of Turn-Around Time 47
	3.2.2	Assay Validation and Reference Compounds 47
	3.2.3	Requirements of Profiling Assay Quality 48
	3.2.4	The Importance of Follow-Up Assays 48
	3.3	Exposure Assays 49
	3.3.1	Basic Absorption Assays 49
	3.3.1.1	Solubility Assays 50
	3.3.1.2	Permeability Assays 50
	3.3.2	Active Transports and Efflux 51
	3.3.3	Metabolism 51
	3.3.4	Distribution and Elimination 51
	3.3.5	Drug–Drug Interactions 53

3.3.6	iviv Correlations 53
3.4	Iterative Assays: Link Between Assays 54
3.5	Specific Safety Profiling Assays 56
3.5.1	Sensitivity and Specificity of Safety Assays should be Adjusted
3.3.1	to the Phase of Drug Discovery 58
3.5.2	Addressing Species Specificity in Early In Vitro Assays 58
3.6	Data Reporting and Data Mining 59
3.6.1	Decision Making: Trend Analysis, Go/No Go Decisions 60
3.7	
3./	Integrative Risk Assessment 61 References 64
	References 04
	Part II
4	Solubility and Aggregation 71
	William H. Streng
4.1	Importance of Solubility 71
4.2	Factors Influencing Solubility 72
4.3	Methods Used to Determine Solubility 74
4.4	Approaches to Solubility 76
4.5	Solubility in Non-Aqueous Solvents and Co-Solvents 78
4.6	Solubility as a Function of pH 79
4.7	Effect of Aggregation Upon Solubility 83
4.8	Dependence of Dissolution upon Solubility 86
4.9	Partitioning and the Effect of Aggregation 87
4.10	Solubility in Simulated Biological Fluids 89
	References 90
5	In Silico Tools and In Vitro HTS Approaches to Determine
_	Lipophilicity During the Drug Discovery Process 91
	Sophie Martel, Vincent Gasparik, and Pierre-Alain Carrupt
5.1	Introduction 91
5.2	Virtual Filtering: In Silico Prediction of log P and log D 92
5.2.1	Lipophilicity of Neutral Substances: In Silico Methods to
	Predict log P ^N _{oct} 92
5.2.1.1	2D Fragmental Approaches 92
5.2.1.2	Prediction Methods Based on 3-D Molecular Structure 95
5.2.1.3	General Comments on the Prediction of log Poct 96
5.2.2	Prediction Models for log P in Other Solvent/Water Systems of
	Neutral Compounds 97
5.2.3	Prediction Models for log P of Ionic Species (log P ^I) 97
5.3	Experimental Filtering: the ADMET Characterization of a
	Hit Collection 98
5.3.1	HTS log P/log D Determination Based on Microtiterplate Format 98
5.3.2	Chromatographic Methods 100
	•

VIII	Contents	
	5.3.2.1	Reverse-Phase Liquid Chromatography 100
	5.3.2.2	Immobilized Artificial Membranes 102
	5.3.2.3	Hydrophilic Interaction Chromatography 103
	5.3.2.4	Capillary Electrophoresis 104
	5.3.3	A Global View On <i>In Vitro</i> HTS Methods to Measure log P/log D 104
	5.4	Concluding Remarks: Efficacy or Accuracy Dilemma 105 References 107
	6	Membrane Permeability – Measurement and Prediction in Drug Discovery 117 Kiyohiko Sugano, Lourdes Cucurull-Sanchez, and Joanne Bennett
	6.1	Overview of Membrane Permeation 117
	6.1.1	Structure, Physiology and Chemistry of the Membrane 117
	6.1.2	Passive Transcellular Pathway: pH Partition Theory as the Basis of Understanding Membrane Permeability 118
	6.1.3	Paracellular Pathway 119
	6.1.4	Active Transporters 119
	6.1.5	In Vitro-In Vivo Extrapolation 119
	6.2	In Vitro Cell Models 121
	6.2.1	Intestinal Cell Culture Models 121
	6.2.2	BBB Cell Culture Models 122
	6.2.3	Cell Models to Study Active Transporters 123
	6.2.4	Correlation of <i>in Vitro</i> Models to Human P_{eff} and Fraction Absorbed Data 124
	6.2.5	Correlation of Cell Culture Models with <i>In Vivo</i> Brain Penetration 124
	6.3	Artificial Membranes 125
	6.3.1	Partition and Permeation 125
	6.3.2	Parallel Artificial Membrane Permeation Assay: Recent Progress 126
	6.3.2.1	Understanding PAMPA 126
	6.3.2.2	Variation of PAMPA: Recent Progress 127
	6.3.2.3	Phospholipid Vesicle PAMPA 127
	6.3.2.4	Phospholipid-Octanol PAMPA 127
	6.3.2.5	Tri-Layer PAMPA 127
	6.3.2.6	Mucus Layer Adhered PAMPA 127
	6.3.3	Application of PAMPA for Drug Discovery 128
	6.4	Limitation of In Vitro Assays 128
	6.4.1	Impact of UWL on Permeability 128
	6.4.2	Membrane Binding 129
	6.4.3	Low Solubility 129
	6.4.4	Difference of the Paracellular Pathway 129
	6.4.5	Interlaboratory Variability 129
	6.5	Computational Approaches/In Silico Modeling 130

6.5.1	In Vivo Systems 130
6.5.2	In Vitro Cellular Membrane Systems 132
6.5.3	Artificial Membranes 134
6.5.4	Perspectives 135
6.6	Outlook 135
	References 136
7	Drug Metabolism and Reactive Metabolites 145 Alan P. Watt
7.1	Introduction to Drug Metabolism 145
7.1.1	Historical Perspective 145
7.1.2	In Vitro Metabolism 146
7.1.2	Cytochrome P450 148
7.1.3	Prediction of Drug Metabolism 149
7.1.4	Adverse Drug Reactions 149
7.2.1	ADR Classification 150
7.2.1	
	Idiosyncratic Drug Reactions 150
7.3	Bioactivation 151
7.3.1	Definition 151
7.3.2	Reactions of Electrophilic Metabolites 151
7.3.3	Glutathione 151
7.3.4	Detection of GSH Conjugates 151
7.3.5 7.3.6	Acyl Glucuronides 152
,	Free Radicals and Oxidative Stress 152
7.4	Reactive Metabolites and Idiosyncratic Toxicity 153
7.4.1	The Hapten Hypothesis 153
7.4.1.1	Immune-Mediated Cutaneous Reactions 153
7.4.2	The Danger Hypothesis 153
7.4.3	Alternate Perspectives to Covalent Binding 154
7.4.3.1	Non-Toxicological Covalent Binding 154
7.4.3.2	Covalent Binding as Detoxification 154
7.5	Measurement of Reactive Metabolites 155
7.5.1	Trapping Assays 155
7.5.1.1	Soft Nucleophiles 155
7.5.1.2	Hard Nucleophiles 155
7.5.2	Mass Spectrometric Detection of GSH Conjugates and
	Mercapturic Acids 155
7.5.3	Radiometric Assays 156
7.5.3.1	Covalent Binding to Liver Microsomes 157
7.5.3.2	Ex Vivo Covalent Binding 157
7.5.3.3	¹⁴ C Cyanide Trapping 157
7.5.3.4	Radiolabeled Soft Nucleophile Trapping 158
7.5.4	Alternate Approaches 158
7.6	Strategies for Minimizing Reactive Metabolite Risk 159
7.6.1	Dose and Exposure 159

x	Contents	
	7.6.2	Structural Alerts 159
	7.6.3	Cascade for Radiolabeled Covalent Binding Experiments 160
	7.6.4	Criteria for Progression 160
	7.7	Conclusions 160
		References 161
	8	Drug-Drug Interactions: Screening for Liability and Assessment of Risk 165
		Ruth Hyland, R. Scott Obach, Chad Stoner, Michael West,
		Michael R. Wester, Kuresh Youdim, and Michael Zientek
	8.1	Introduction 165
	8.2	In Silico Approaches 167
	8.3	Perpetrators of Drug-Drug Interactions: Enzyme Inhibition 169
	8.3.1	Competitive Inhibition 169
	8.3.2	Conventional CYP Inhibition Screen 170
	8.3.3	Fluorescent Inhibition Screen 172
	8.3.4	DDI Single Point versus IC ₅₀ Determinations 172
	8.3.5	DDI Cocktail Assay 173
	8.3.6	Mechanism-Based Inhibition 174
	8.4	Perpetrators of Drug-Drug Interactions: Enzyme Induction 176
	8.4.1	Ligand Binding Assay 177
	8.4.2	Reporter Gene (Transactivation) Assays 178
	8.4.3	Overall Evaluation of High-Throughput Induction Assays 179
	8.5	Drug-Drug Interactions; Victims of Interaction; Reaction
		Phenotyping 179
	8.5.1	Chemical Inhibition 180
	8.5.2	Recombinant Human CYP Enzymes 181
	8.6	Predictions of Drug-Drug Interactions 182
	8.6.1	New Compounds as Potential DDI Perpetrators 183
	8.6.2	New Compounds as Potential DDI Victims 184
	8.7	Summary 187
		References 188
	9	Plasma Protein Binding and Volume of Distribution: Determination,
		Prediction and Use in Early Drug Discovery 197
		Franco Lombardo, R. Scott Obach, and Nigel J. Waters
	9.1	Introduction: Importance of Plasma Protein Binding 197
	9.2	Impact of Plasma Protein Binding on PK, Exposure, Safety Margins,
		Potency Screens and Drug-Drug Interaction 197
	9.3	Methodologies for Measuring Plasma Protein Binding 201
	9.4	Physicochemical Determinants and In Silico Prediction of Plasma
		Protein Binding 206
	9.5	Volume of Distribution: General Considerations and Applications to
		Experimental Pharmacokinetics and Drug Design 208
	9.5.1	Prediction of Human Volume of Distribution 210

9.5.1.1	Prediction of Human Volume of Distribution from Animal Pharmacokinetic Data 210
9.5.1.2	Prediction of Human Volume of Distribution from
9.5.1.3	In Vitro Data 212 Production of Human Volume of Distribution from In Cilia
9.3.1.3	Prediction of Human Volume of Distribution from <i>In Silico</i> Methods 213
9.6	Relationship Between Clearance, VDss and Plasma Protein
	Binding 213
9.7	Summary and Conclusions 214
	References 215
10	Putting It All Together 221
	Pamela Berry, Neil Parrott, Micaela Reddy, Pascale David-Pierson,
	and Thierry Lavé
10.1	Challenges in Drug Discovery 221
10.2	Methodological Aspects 222
10.2.1	PBPK 222
10.2.2	PK/PD 225
10.3	Strategic Use of PBPK During Drug Discovery 226
10.4	Strategic Use of PK/PD During Drug Discovery 227
10.5	Application During Lead Identification 227
10.6	Application During Lead Optimization 232
10.7	Application During Clinical Lead Selection 235
10.8	Limitations with Current Methodology and Approaches 236
10.9	Conclusions 238
	References 238
	Part III
11	Genetic Toxicity: In Vitro Approaches for Hit and Lead Profiling 243
	Richard M Walmsley and Nicholas Billinton
11.1	Introduction 243
11.2	Definitions 245
11.3	Major Challenges for Early, Predictive Genotoxicity Testing 246
11.4	Practical Issues for Genotoxicity Profiling: Vehicle, Dose, Dilution
	Range and Impurity 248
11.4.1	Vehicle and Dose 248
11.4.2	Dilution Range 249
11.4.3	Purity 249
11.5	Computational Approaches to Genotoxicity Assessment: "In Silico"
	Assessment 250
11.5.1	How Should In Silico Methods be Applied in Hit and Lead Profiling? 252
11.6	Genotoxicity Assays for Screening 253
11.6.1	Gene Mutation Assays 254

ХII	Contents	
	11.6.2	The Ames Test and Variants 255
	11.6.3	Mammalian Cell Mutation Assays 256
	11.6.4	Saccharomyces cerevisiae ("Yeast") Mutation Assays 256
	11.7	Chromosome Damage and Aberration Assays 256
	11.7.1	Aberrations 256
	11.7.2	Micronuclei 257
	11.7.3	"Comet" Assay 258
	11.7.4	DNA Adduct Assessment 258
	11.7.5	Gene Expression Assays 259
	11.7.5.1	Prokaryotic 259
	11.7.5.2	Eukaryotic 259
	11.8	Using Data from <i>In Vitro</i> Profiling: Confirmatory Tests, Follow-Up Tests, and the Link to Safety Assessment and <i>In Vivo</i> Models 260
	11.8.1	Annotations from Screening Data 261
	11.8.2	Annotations from Positive Screening Data 262
	11.8.2.1	Gene Mutation Assays 262
	11.8.2.2	Chromosome Damage Assays 262
	11.8.2.3	Reporter Assays 263
	11.9	Can a Genetic Toxicity Profile Inform In Vivo Testing Strategies? 263
	11.9.1	Prospects for In Vivo Profiling of Hits and Leads for Genotoxicity 264
	11.10	What to Test, When and How? 265
	11.10.1	Profiling Entire Libraries: >100 000 Compounds/Year 265
	11.10.2	Profiling Hits: 10 000-100 000 Compounds/Year 265
	11.10.3	Profiling in Lead Optimization: 2000-10 000 Compounds/Year 266
	11.11	Summary 267
		References 267
	12	In Vitro Safety Pharmacology Profiling: an Important Tool to Decrease Attrition 273 Jacques Hamon and Steven Whitebread
	12.1	What is "In Vitro Safety Pharmacology Profiling?" 273
	12.1	Examples of Drug Failures Due to Secondary Pharmacology 274
	12.2.1	-
	12.2.1.1	-
	12.2.1.1	Target Selection 275 Target Annotation 276
	12.2.1.3	Examples of <i>In Vitro</i> Safety Pharmacology Profiling Panels 277
	12.2.1.3	Processes 280
	12.3.1	Assay Requirements and Technologies 280
	12.3.1	Binding and/or Functional Assays 284
	12.3.3	Processes and Logistics 286

Application to Drug Discovery 287

Profiling 287

How and When to Use In Vitro Safety Pharmacology

Relevance of Potency and Therapeutic Index (TI) 290

Pharmacological Promiscuity and Its Clinical Interpretation

288

12.4

12.4.1

12.4.2

12.4.3

	Contents XIII
12.4.4 12.5	Possible Benefits of Off-Target Effects 291 Conclusions and Outlook 291 References 292
	References 292
13	Knowledge-Based and Computational Approaches to In Vitro Safety
	Pharmacology 297
13.1	Josef Scheiber, Andreas Bender, Kamal Azzaoui, and Jeremy Jenkins Introduction 297
13.1 13.1.1	The Value of Safety Pharmacology Data: the Value and Relevance
13.1.1	of Complete, Standardized Data Matrices for <i>In Silico</i> Prediction of Adverse Events 298
13.2	"Meta Analysis" of Safety Pharmacology Data: Predicting Compound
	Promiscuity 304
13.2.1	Introduction 304
13.2.2	Data Analysis 305
13.2.2.1	Hit Rate Parameter and Chemical Profiling 305
13.2.2.2	Computational Efforts: Generation of Hypotheses 307
13.2.2.3	Promiscuity and Attrition Rate 308
13.2.2.4	Conclusion on Promiscuity Prediction 310
13.3	Prediction of Off-Target Effects of Molecules Based on Chemical Structure 310
13.3.1	Introduction 310
13.3.2	Available Databases and Desired Format 311
13.3.3	The Best Established Technologies for In Silico Target Fishing 313
13.3.3.1	Similarity Searching in Databases 313
13.3.3.2	Data Mining in Annotated Chemical Databases 314
13.3.3.3	Data Mining on Bioactivity Spectra 314
13.4	Future Directions 316
	References 317
	Part IV
14	Discovery Toxicology Screening: Predictive, In Vitro Cytotoxicity 325
, ,	Peter J. O'Brien
14.1	Introduction 325
14.2	Basis of Need for Discovery Toxicology Screening 326
14.2.1	High Attrition at High Cost 326
14.2.2	High Proportion of Attrition Due to Adverse Safety 326
14.2.3	Discovery Screening Reduces Attrition by An Order of Magnitude 326
14.3	Obstacles to Discovery Toxicology Screening 327
14.4	Need to Coordinate Cytotoxicity Screening with Other Discovery
	Safety Assessments 327
14.5	Discovery Cytotoxicology 329
14.5.1	Biomarkers for Safety versus Efficacy for Screening 329

χιν	Contents	
	14.5.2	Past Failure of Cytotoxicity Assessments 329
	14.5.2.1	Insufficient Exposure 329
	14.5.2.2	Measurement of Cell Death 330
	14.5.3	Effective Cell-Based Assays for Marked and Acute Cytotoxicity 331
	14.5.4	Characteristics of an Optimally Effective Cell Model of Toxicity 331
	14.5.4.1	Need for Morphological and Functional Parameters 333
	14.5.4.2	Need for Multiple and Mechanistic Parameters 333
	14.5.4.3	Need for Single-Cell Monitoring 333
	14.5.4.4	Need for Effective Parameters 334
	14.5.4.5	Need for Validation with Human Toxicity Data 336
	14.6	High Effectiveness of an HCA Cell Model in Predictive Toxicology 337
	14.6.1	Background on HCA 337
	14.6.2	Idiosyncratic Hepatotoxicity 337
	14.6.3	Characteristic Pattern and Sequence of Cytotoxic Changes 338
	14.6.4	Safety Margin 338
	14.6.5	Hormesis 338
	14.6.6	Implementation of HCA Cytotoxicity Testing in Drug Discovery 339
	14.6.7	Limitations of HCA Cytotoxicity Testing in Drug Discovery 340
	14.7	Future Impact of Cytotoxicity Testing 340
		References 341
	15	Predicting Drug-Induced Hepatotoxicity: In Vitro, In Silico and
		In Vivo Approaches 345
		Jinghai J. Xu, Amit S. Kalgutkar, Yvonne Will, James Dykens,
		Elizabeth Tengstrand, and Frank Hsieh
	15.1	Introduction 345
	15.2	Reactive Metabolites 346
	15.2.1	Assays and In Silico Knowledge to Assess Bioactivation Potential 347
	15.2.1.1	In Vitro Reactive Metabolite Trapping Studies 347
	15.2.1.2	Covalent Binding Determinations 348
	15.2.2	Utility of Reactive Metabolite Trapping and Covalent Binding Studies
		in Drug Discovery 348
	15.2.3	Are Reactive Metabolite Trapping and Covalent Binding Studies
		Reliable Predictors of Hepatotoxic Potential of Drug Candidates? 348
	15.2.4	Mitigating Factors Against Hepatotoxicity Risks Due to Bioactivation –
		a Balanced Approach Towards Candidate Selection in Drug Discovery 351
	15.2.5	Future Directions 355
	15.3	Mitochondrial Toxicity 356
	15.3.1	Uncouplers of Mitochondrial Respiration 358
	15.3.2	Drugs that Inhibit OXPHOS Complexes 358

Drugs that Induce the Mitochondrial Permeability Transition Pore

Drugs Inhibiting mtDNA Synthesis and Mitochondrial Protein

Inhibition of Fatty Acid β-Oxidation or Depletion of CoA 360

15.3.3

15.3.4

15.3.5

(MPT) 359

Synthesis 359

15.3.6	In Vitro and In Vivo Assessment of Drug-Induced Mitochondrial Dysfunction 360
15.4	Oxidative Stress 363
15.4.1	Sources of Oxidative Stress 363
15.4.2	Measurements of Oxidative Stress 363
15.4.3	Critical Review: Is There Sufficient Clinical, Pre-Clinical and
13.1.3	In Vitro Data to Substantiate the Link Between Oxidative Stress and Idiosyncratic Liver Injury? 364
15.5	Inhibition of Bile Salt Efflux Protein and Drug-Induced Cholestasis 365
15.5.1	In Vitro and In Vivo Assays to Measure BSEP Inhibition 365
15.5.2	Critical Review: Is There a Link between BSEP Inhibition, Drug-Induced
	Cholestasis and Idiosyncratic Liver Injury? 368
15.6	Biomarkers 369
15.6.1	Hepatocellular Injury 370
15.6.2	Cholestatic Injury 370
15.6.3	Application of Serum Chemistry Markers 370
15.6.4	Need for New Biomarkers 371
15.6.5	Biomarker Discovery Efforts 372
15.6.6	Approaches for Biomarker Discovery 372
15.6.6.1	Development of In Vivo Biomarkers 373
15.6.6.2	Development of In Vitro Biomarkers 373
15.6.6.3	Biomarker Validation 374
15.6.7	Future Biomarker Directions 374
15.7	Conclusions 375
	References 376
16	Should Cardiosafety be Ruled by hERG Inhibition?
	Early Testing Scenarios and Integrated Risk Assessment 387
	Dimitri Mikhailov, Martin Traebert, Qiang Lu, Steven Whitebread,
	and William Egan
16.1	Introduction 387
16.2	Role of Ion Channels in Heart Electrophysiology 389
16.3	hERG Profiling Assays 391
16.3.1	Cell-Free Competition Binding Assays 392
16.3.1.1	Radioligand Binding 393
16.3.1.2	Fluorescence Polarization 393
16.3.2	Non-Electrophysiological Functional Cellular Assays 393
16.3.2.1	Rubidium Efflux and Thallium Influx 393
16.3.2.2	Membrane Potential-Sensitive Fluorescent Dyes 394
16.3.3	Higher-Throughput Planar Patch Technologies 394
16.3.4	Non-hERG Ion Channel Assays Related to Cardiotoxicity 395
16.3.5	Nonclinical Cardiosafety Assays in Early Drug Development 396
16.4	Computational Models for hERG 398
16.4.1	Pharmacophore Models 398
16.4.2	Docking to Homology Models 399

xvı	Contents	
	16.4.3	QSAR Models 400
	16.5	Integrated Risk Assessment 401
	16.5.1	Cardiosafety Assessment of Early Discovery Projects 401
	16.5.2	Cardiosafety Assessment of Preclinical Positive Signals 403
	16.6	Summary 405
		References 406
	17	Hematotoxicity: In Vitro and Ex Vivo Compound Profiling 415
		David Brott and Francois Pognan
	17.1	Introduction 415
	17.2	Known Compounds with Hematotoxic Potential 417
	17.3	Tiered Cascade of Testing 419
	17.3.1	Tier 1 Tests 420
	17.3.2	Tier 2 Tests 426
	17.3.3	Tier 3 Tests 428
	17.4	Triggers for Hematotoxicity Testing 430
	17.5	Conclusions 433
		References 433
	18	Profiling Adverse Immune Effects 439
		Wim H. De Jong, Raymond Pieters, Kirsten A Baken, Rob J. Vandebriel,
		Jan-Willem Van Der Laan, and Henk Van Loveren
	18.1	Immunotoxicology 439
	18.1.1	The Immune System and Immunotoxicology 439
	18.1.2	Detection of Immunotoxicity 442
	18.1.3	Evaluation of the Immune System in Toxicity Studies 443
	18.1.4	Testing for Induction of Allergy 445
	18.1.5	Testing for Induction of Autoimmunity 446
	18.1.5.1	Introduction 446
	18.1.5.2	Assays for Testing the Induction of Autoimmunity 446
	18.1.5.3	Alternative Approach for Evaluation of Autoimmunity Potential of Chemicals 447
	18.1.6	Structures Associated with Immunotoxicity 449
	18.1.7	Immunostimulation by Components of the Immune Systems
		Used as Therapeutics 450
	18.2	Non-Animal Approaches for the Determination of Immunotoxicity 451
	18.2.1	In Silico Approaches 451
	18.2.2	In Vitro Approaches to Test Various Aspects of Immunotoxicity 451
	18.2.2.1	Introduction 451
	18.2.2.2	Immunosuppression 453
	18.2.2.3	Chemical Sensitization 454
	18.2.2.4	Conclusions 456
	18.2.3	Toxicogenomics 456
	18.2.3.1	Introduction 456
	18.2.3.2	Immunotoxicogenomics 456

18.2.3.3	Interpretation of Results 457
18.2.3.4	Toxicogenomics for Prediction of Effects 457
18.2.3.5	Target Organs and Cells for Immunotoxicity 458
18.2.3.6	Conclusions 458
18.3	Summary 459
	References 459
19	In Vitro Phototoxicity Testing: a Procedure Involving Multiple
	Endpoints 471
	Laurent Marrot and Jean-Roch Meunier
19.1	Introduction 471
19.2	Optical Considerations: Relevant UV Sources and Sunlight
	Absorption 472
19.2.1	Working with the Appropriate Artificial Sunlight Source Determines the Relevance of Phototoxicity Screening 472
19.2.2	When to Study the Phototoxicity of a Substance? 474
19.3	In Silico Methods for Prediction of Phototoxicity – (Q)SAR Models 474
19.3.1	Global Models 475
19.3.2	Local Models 475
19.4	Photoreactivity In Tubo: Prescreening of Compounds Producing
	ROS Upon Sunlight Exposure 478
19.4.1.	Biochemical Detection of Photoinduced ROS 478
19.4.2	Photo-Cleavage of Isolated Plasmid DNA 479
19.4.3	Photo Red Blood Cells Test 479
19.5	Microbiological Models for Photomutagenesis Assessment 480
19.5.1	Photo-Ames Test 480
19.5.2	The Yeast Model 480
19.6	Photocytotoxicity and Photogenotoxicity in Mammalian Cells:
	Regulatory Tests and Beyond 482
19.6.1	The 3T3 NRU Assay: a Validated Test for the Assessment of a
	Photoirritation Potential 482
19.6.2	Photogenotoxicity: an Endpoint Without Corresponding In Vivo
	Equivalents 483
19.7	Reconstructed Skin: a Model for Mimicking Phototoxicity in the
	Target Organ 486
19.8	Conclusions 488
	References 489

Index 495