CONTENTS

PREFACE xxxv
ABOUT THE AUTHOR xxvii

1 The Drug Development Process and the Global Pharmaceutical Marketplace 1
  1.1 Introduction, 1
  1.2 The Marketplace, 1
  1.3 History of Modern Therapeutics, 4
  1.4 The Drug Development Process, 6
  1.5 Strategies for Development: Large Versus
      Small Company or the Short Versus Long Game, 7
  1.5.1 Do Only What You Must, 8
  1.5.2 Minimize the Risk of Subsequent Failure, 9
  1.6 Safety Assessment and the Evolution of Drug Safety, 11
  1.7 The Three Stages of Drug Safety Evaluation in the General Case, 11
  References, 12

2 Regulation of Human Pharmaceutical Safety: Routes to
   Human Use and Market 13
  2.1 Introduction, 13
  2.2 Brief History of US Pharmaceutical Law, 13
     2.2.1 1906: Pure Food and Drug Act, 13
     2.2.2 1938: Food, Drug, and Cosmetic Act, 15
     2.2.3 1962: Major Amendment, 17
  2.3 FDAMA Summary: Consequences and Other Regulations, 19
  2.4 Overview of US Regulations, 21
     2.4.1 Regulations: General Considerations, 21
     2.4.2 Regulations: Human Pharmaceuticals, 22
     2.4.3 Regulations: Environmental Impact, 23
     2.4.4 Regulations: Antibiotics, 23
     2.4.5 Regulations: Biologics, 24
     2.4.6 Regulations versus Law, 24
CONTENTS

2.5 Organizations Regulating Drug and Device Safety in the United States, 24
2.6 Process of Pharmaceutical Product Development and Approval, 25
2.7 Testing Guidelines, 28
  2.7.1 Toxicity Testing: Traditional Pharmaceuticals, 28
  2.7.2 General or Systematic Toxicity Assessment, 28
  2.7.3 Genetic Toxicity Assessment, 28
  2.7.4 Safety Pharmacology, 30
  2.7.5 Local Tissue Tolerance, 30
  2.7.6 Toxicity Testing: Biotechnology Products, 31
2.8 Toxicity/Safety Testing: Cellular and Gene Therapy Products, 33
  2.8.1 Cellular Therapies, 34
  2.8.2 Gene Therapies, 34
  2.8.3 Ex Vivo, 34
  2.8.4 In Vivo, 34
  2.8.5 Preclinical Safety Evaluation, 34
  2.8.6 Basic Principles for Preclinical Safety Evaluation
       of Cellular and Gene Therapies, 35
  2.8.7 Additional Considerations for Cellular Therapies, 35
  2.8.8 Additional Considerations for Gene Therapies, 35
2.9 Toxicity Testing: Special Cases, 35
  2.9.1 Oral Contraceptives, 35
  2.9.2 Life‐Threatening Diseases (Compassionate Use), 35
  2.9.3 Optical Isomers, 36
  2.9.4 Special Populations: Pediatric and Geriatric Claims, 37
  2.9.5 Orphan Drugs, 38
  2.9.6 Botanical Drug Products, 41
  2.9.7 Types of New Drug Applications (NDAs), 41
2.10 International Pharmaceutical Regulation and Registration, 41
  2.10.1 International Conference on Harmonization, 41
  2.10.2 Other International Considerations, 45
  2.10.3 Safety Pharmacology, 50
2.11 Combination Products, 50
  2.11.1 Device Programs That CDER and CBRH Each Will Administer, 51
  2.11.2 Coordination, 51
  2.11.3 Submissions, 51
2.12 Conclusions, 55
References, 55
Further Reading, 57

3 Data Mining: Sources of Information for Consideration
in Study and Program Design and in Safety Evaluation 59

3.1 Introduction, 59
  3.1.1 Claims, 59
  3.1.2 Time and Economies, 59
  3.1.3 Prior Knowledge, 59
  3.1.4 Miscellaneous Reference Sources, 60
  3.1.5 Search Procedure, 62
  3.1.6 Monitoring Published Literature and Other Research in Progress, 62
  3.1.7 Kinds of Information, 63
  3.1.8 Toxic Release Inventory (TRI), 63
  3.1.9 Material Safety Data Sheets (MSDS), 63
  3.1.10 Canadian Centre for Occupational Health and Safety (CCINFO), 64
  3.1.11 Pollution and Toxicology (POLTOX), 64
  3.1.12 MEDLINE, 64
3.2 PC-Based Information Products: Laser DISC, 65
   3.2.1 International Veterinary Pathology Slide Bank (IVPSB), 65
3.3 Conclusions, 65
References, 65

4 Screens in Safety and Hazard Assessment 67
   4.1 Introduction, 67
   4.2 Characteristics of Screens, 68
   4.3 Uses of Screens, 70
   4.4 Types of Screens, 71
      4.4.1 Single Stage, 71
      4.4.2 Sequential, 71
      4.4.3 Tier (or Multistage), 71
   4.5 Criterion: Development and Use, 71
   4.6 Analysis of Screening Data, 73
   4.7 Univariate Data, 73
      4.7.1 Control Charts, 73
      4.7.2 Central Tendency Plots, 74
      4.7.3 Multivariate Data, 75
      4.7.4 The Analog Plot, 75
References, 76

5 Formulations, Routes, and Dosage Regimens 79
   5.1 Mechanisms, 81
      5.1.1 Local Effects, 81
      5.1.2 Absorption and Distribution, 81
      5.1.3 Metabolism, 82
   5.2 Common Routes, 83
      5.2.1 Dermal Route, 83
      5.2.2 Parenteral Route, 84
      5.2.3 Bolus versus Infusion, 85
      5.2.4 Oral Route, 86
      5.2.5 Minor Routes, 94
      5.2.6 Route Comparisons and Contrasts, 96
   5.3 Formulation of Test Materials, 96
      5.3.1 Preformulation, 97
      5.3.2 Dermal Formulations, 100
      5.3.3 Interactions between Skin, Vehicle, and Test Chemical, 102
      5.3.4 Oral Formulations, 103
      5.3.5 Parenteral Formulations, 104
   5.4 Dosing Calculations, 105
   5.5 Calculating Material Requirements, 105
   5.6 Excipients, 106
      5.6.1 Regulation of Excipients, 106
References, 111

6 Nonclinical Manifestations, Mechanisms, and End Points of Drug Toxicity 115
   6.1 Manifestations, 115
   6.2 Mechanisms of Toxicity, 116
   6.3 End Points Measured in General Toxicity Studies, 116
      6.3.1 Clinical Observations, 116
      6.3.2 Body Weights, 116
      6.3.3 Food and Water Consumption, 116
6.3.4 Clinical Signs, 117
6.3.5 Clinical Chemistry and Pathology, 117
6.3.6 Hematology, 124
6.3.7 Gross Necropsy and Organ Weights, 124
6.3.8 Histopathology, 125
6.3.9 Ophthalmology, 125
6.3.10 Cardiovascular Function, 125
6.3.11 Neurotoxicology, 125
6.3.12 Immunotoxicology, 125
6.4 Complications, 126
References, 126

7 Pilot Toxicity Testing in Drug Safety Evaluation: MTD and DRF 129
7.1 Introduction, 129
7.2 Range-Finding Studies, 130
  7.2.1 Lethality Testing, 130
  7.2.2 Using Range-Finding Lethality Data in Drug Development: The Minimum Lethal Dose, 136
7.3 Acute Systemic Toxicity Characterization, 138
  7.3.1 Minimal Acute Toxicity Test, 139
  7.3.2 Complete Acute Toxicity Testing, 142
  7.3.3 Acute Toxicity Testing with Nonrodent Species, 146
  7.3.4 Factors that Can Affect Acute Tests, 148
  7.3.5 Selection of Dosages, 149
7.4 Screens, 150
  7.4.1 General Toxicity Screens, 151
  7.4.2 Specific Toxicity Screening, 153
7.5 PILOT and DRF Studies, 154
References, 156

8 Repeat-Dose Toxicity Studies 159
8.1 Objectives, 159
8.2 Regulatory Considerations, 161
  8.2.1 Good Laboratory Practices, 161
  8.2.2 Animal Welfare Act, 161
  8.2.3 Regulatory Requirements for Study Design, 162
8.3 Study Design and Conduct, 162
  8.3.1 Animals, 162
  8.3.2 Routes and Setting Doses, 163
  8.3.3 Parameters to Measure, 164
  8.3.4 Study Designs, 164
8.4 Study Interpretation and Reporting, 165
References, 166

9 Genotoxicity 169
9.1 ICH Test Profile, 169
9.2 DNA Structure, 169
  9.2.1 Transcription, 171
  9.2.2 Translation, 171
  9.2.3 Gene Regulation, 171
  9.2.4 DNA Repair, 171
  9.2.5 Error-Prone Repair, 172
  9.2.6 Mismatch Repair, 172
  9.2.7 The Adaptive Repair Pathway, 172
  9.2.8 Plasmids, 172
  9.2.9 Plasmids and DNA Repair, 173
9.2.10 Nature of Point Mutations, 173
9.2.11 Suppressor Mutations, 173
9.2.12 Adduct Formation, 173
9.2.13 Mutations Due to Insertion Sequences, 174
9.2.14 The Link between Mutation and Cancer, 174
9.2.15 Genotoxic versus Nongenotoxic Mechanisms of Carcinogenesis, 174
9.2.16 Genetic Damage and Heritable Defects, 175
9.2.17 Reproductive Effects, 176
9.3 Cytogenetics, 176
9.3.1 Cytogenetic Damage and Its Consequences, 176
9.3.2 Individual Chromosomal Damage, 176
9.3.3 Chromosome Set Damage, 177
9.3.4 Test Systems, 177
9.3.5 In Vitro Test Systems, 178
9.3.6 Bacterial Mutation Tests, 180
9.3.7 Controls, 182
9.3.8 Plate Incorporation Assay, 184
9.3.9 Eukaryotic Mutation Tests, 185
9.3.10 In Vitro Tests for the Detection of Mammalian Mutation, 185
9.3.11 In Vivo Mammalian Mutation Tests, 193
9.4 In Vitro Cytogenetic Assays, 193
9.4.1 Cell Types, 194
9.4.2 Chinese Hamster Cell Lines, 194
9.4.3 Human Peripheral Blood Lymphocytes, 194
9.4.4 Positive and Negative Controls, 194
9.4.5 Treatment of Cells, 195
9.4.6 Scoring Procedures, 195
9.4.7 Data Recording, 196
9.4.8 Presentation of Results, 196
9.5 In Vivo Cytogenetic Assays, 196
9.5.1 Somatic Cell Assays, 196
9.5.2 Germ Cell Assays, 197
9.5.3 Heritable Chromosome Assays, 197
9.5.4 Germ Cell Cytogenetic Assays, 197
9.6 Sister Chromatid Exchange Assays, 197
9.6.1 Relevance of SCE in Terms of Genotoxicity, 198
9.6.2 Experimental Design, 198
References, 199

10 QSAR Tools for Drug Safety

10.1 Structure–Activity Relationships, 209
10.1.1 Basic Assumptions, 210
10.1.2 Molecular Parameters of Interest, 210
10.2 SAR Modeling Methods, 210
10.3 Applications in Toxicology, 212
10.3.1 Metabolism, 213
10.3.2 Reproductive, 213
10.3.3 Eye Irritation, 213
10.3.4 Lethality, 214
10.3.5 Carcinogenicity, 214
10.4 Genotoxicity, 215
10.4.1 QSAR for Mutagenicity, 215
10.5 Comparison of Available Models/Applications, 216
10.5.1 QSAR of Metabolism, 216
CONTENTS

10.5.2 Meteor, 216
10.5.3 Derek, 218
10.5.4 Leadscope, 219
10.5.5 VEGA, 219
10.5.6 Derek versus Leadscope, 222
References, 222

11 Immunotoxicology in Drug Development 225
11.1 Introduction, 225
11.2 Overview of the Immune System, 227
11.3 Immunotoxic Effects, 229
11.4 Immunosuppression, 231
11.4.1 Immunosuppressive Drugs, 232
11.5 Immunostimulation, 235
11.5.1 Hypersensitivity (or Allergenicity), 235
11.5.2 Photosensitization, 238
11.5.3 Autoimmunity, 238
11.6 Regulatory Positions, 240
11.6.1 CDER Guidance for Investigational New Drugs, 242
11.7 Evaluation of the Immune System, 245
11.7.1 Immunopathologic Assessments, 246
11.7.2 Humoral (Innate) Immune Response and Possible Entry Points for Immunotoxic Actions, 246
11.7.3 Cell‐Mediated Immunity, 250
11.8 Nonspecific Immunity Function Assay, 251
11.8.1 Natural Killer Cell Assays, 251
11.8.2 Macrophage Function, 251
11.8.3 Mast Cell/Basophil Function, 252
11.9 T‐Cell‐Dependent Antibody Response (TDAR), 253
11.9.1 Treatment, 253
11.9.2 Hypersensitivity, 253
11.9.3 Local Lymph Node Assay (LLNA), 255
11.9.4 Photosensitization, 258
11.10 Approaches to Compound Evaluation, 259
11.10.1 Use of In Vivo Tests, 260
11.10.2 Use of In Vitro Tests, 261
11.10.3 Assessment of Immunotoxicity and Immunogenicity/Allergenicity of Biotechnology‐Derived Drugs, 261
11.10.4 Suggested Approaches to Evaluation of Results, 262
11.11 Problems and Future Directions, 263
11.11.1 Data Interpretation, 263
11.11.2 Appropriate Animal Models, 263
11.11.3 Indirect Immunotoxic Effects, 263
11.11.4 Hypersensitivity Tests, 263
11.11.5 Autoimmunity, 264
11.11.6 Functional Reserve Capacity, 264
11.11.7 Significance of Minor Perturbations, 264
11.11.8 Biotechnology Products, 264
References, 264

12 Nonrodent Animal Studies 269
12.1 Introduction, 269
12.2 Comparison Between Rodent and Nonrodent Experimental Design, 269
12.2.1 Number of Animals, 269
References, 264
12.3 Differences in Study Activities, 270
  12.3.1 Blood Collection, 270
  12.3.2 Dosing, 270
  12.3.3 Handling of Animals, 270
  12.3.4 Behavioral Evaluation, 270
12.4 Nonrodent Models, 270
12.5 Dog, 270
  12.5.1 Environmental and Dietary Requirements, 270
  12.5.2 Common Study Protocols, 271
  12.5.3 General Study Activities, 272
  12.5.4 Advantages and Disadvantages, 272
12.6 The Ferret, 273
  12.6.1 Environmental and Dietary Requirements, 273
  12.6.2 Study Protocols, 273
  12.6.3 General Study Activities, 274
  12.6.4 Advantages and Disadvantages, 275
12.7 The Pig, 275
  12.7.1 Background, 275
  12.7.2 Clinical Laboratory, 276
  12.7.3 Xenobiotic Metabolism, 277
  12.7.4 Dermal Toxicity, 278
  12.7.5 Cardiovascular Toxicity, 279
  12.7.6 Advantages and Disadvantages, 279
12.8 Nonhuman Primates, 279
  12.8.1 Environmental and Dietary Requirements, 281
  12.8.2 Common Study Protocols, 281
  12.8.3 General Study Activities, 281
  12.8.4 Advantages and Disadvantages, 283
12.9 Statistics in Large Animal Studies, 283
  12.9.1 Reasons for Small Sample Sizes in Large Animal Toxicology, 284
  12.9.2 Cross-Sectional or Longitudinal Analysis?, 284
  12.9.3 Repeated Measures: Advantages, 284
  12.9.4 Repeated Measures: Disadvantages, 284
  12.9.5 Common Practices in Large Animal Toxicology, 284
  12.9.6 Univariate (Repeated Measures) Techniques: Advantages, 285
  12.9.7 Univariate (Repeated Measures) Techniques: Disadvantages, 285
  12.9.8 Multivariate Techniques: Advantages, 285
  12.9.9 Multivariate Techniques: Disadvantages, 285
  12.9.10 Some Other Design Factors to Be Considered in Analysis, 285
  12.9.11 Covariates: Advantages, 285
  12.9.12 Covariates: Disadvantages, 285
  12.9.13 Missing Values, 288
12.10 Summary, 288
References, 288

13 Developmental and Reproductive Toxicity Testing 291
13.1 Introduction, 291
13.2 ICH Study Designs, 293
  13.2.1 Male and Female Fertility and Early Embryonic Development to Implantation, 294
  13.2.2 Embryo–Fetal Development, 295
  13.2.3 Adverse Effects, 295
13.2.4 Pre- and Postnatal Development, 295
13.2.5 Single-Study and Two-Study Designs for Rodents, 296
13.2.6 Preliminary Studies, 297
13.2.7 Toxicokinetics, 297
13.2.8 Timing of Studies, 297
13.3 Methodological Issues, 298
13.3.1 Control of Bias, 298
13.3.2 Diet, 298
13.3.3 Clinical Pathology, 299
13.3.4 Gravid Uterine Weights, 299
13.3.5 Implant Counts and Determination of Pregnancy, 301
13.3.6 Fetal Examinations, 301
13.3.7 Developmental Signs, 302
13.3.8 Behavioral Tests, 303
13.3.9 Detecting Effects on Male Reproduction, 303
13.4 Developmental Studies in Primates, 303
13.5 Data Interpretation, 304
13.5.1 Use of Statistical Analyses, 304
13.5.2 Potential Hazard Categories of Developmental Toxins, 307
13.5.3 Associations between Developmental and Maternal Toxicity, 308
13.5.4 Assessment of Human Risk, 308
13.6 Juvenile and Pediatric Toxicology, 310
13.7 In Vitro Tests for Developmental Toxicity, 312
13.8 Appraisal of Current Approaches for Determining Developmental and Reproductive Hazards, 316

References, 317

14 Carcinogenicity Studies 321

14.1 Introduction, 321
14.1.1 History of Xenobiotic Carcinogenesis, 322
14.2 Mechanisms and Classes of Carcinogens, 322
14.3 Genotoxic Carcinogens, 322
14.4 Epigenetic Carcinogens, 325
14.5 Regulatory Requirements and Timing, 328
14.6 Species and Strain, 328
14.7 Animal Husbandry, 330
14.8 Dose Selection, 330
14.8.1 Number of Dose Levels, 330
14.8.2 Number of Control Groups, 330
14.8.3 Criteria for Dose Selection, 331
14.9 Group Size, 331
14.10 Route of Administration, 332
14.11 Study Duration, 332
14.12 Survival, 332
14.13 End Points Measured, 333
14.14 Transgenic Mouse Models, 335
14.14.1 The Tg.AC Mouse Model, 335
14.14.2 The Tg.rasH2 Mouse Model, 336
14.14.3 The P53−/− Mouse Model, 336
14.14.4 The XPA−/− Mouse Model, 337
14.15 Interpretation of Results: Criteria for a Positive Result, 338
14.16 Statistical Analysis, 338
14.16.1 Exact Tests, 339
14.16.2 Trend Tests, 340
14.16.3 Life Table and Survival Analysis, 341
14.16.4 Peto Analysis, 341
14.16.5 Methods to Be Avoided, 342
14.16.6 Use of Historical Controls, 342
14.16.7 Relevance to Humans, 342
14.17 Weight-of-Evidence Factors for Consideration in a Carcinogenicity Assessment Document (CAD), 344
14.18 Conclusions, 345
References, 345

15 Histopathology in Nonclinical Pharmaceutical Safety Assessment 351
15.1 Introduction, 351
15.1.1 Pathological Techniques, 354
15.1.2 Organ Weights, 354
15.2 Clinical Pathology, 355
15.2.1 Clinical Chemistry, 355
15.2.2 Target Organ Toxicity Biomarkers, 355
References, 356

16 Irritation and Local Tissue Tolerance in Pharmaceutical Safety Assessment 359
16.1 Introduction, 359
16.2 Factors Affecting Irritation Responses and Test Outcome, 359
16.3 Primary Dermal Irritation (PDI) Test, 360
16.4 Other Nonparenteral Route Irritation Tests, 362
16.5 Ocular Irritation Testing, 362
16.6 Vaginal Irritation, 364
16.7 Acute Primary Vaginal Irritation Study in the Female Rabbit, 365
16.7.1 Repeated-Dose Vaginal Irritation in the Female Rabbit, 365
16.7.2 Repeated-Dose Vaginal Irritation in the Ovariectomized Rats, 366
16.8 Parenteral Irritation/Tolerance, 367
16.8.1 Parenteral Routes, 367
16.8.2 Test Systems for Parenteral Irritation, 368
16.9 Problems in Testing (and Their Resolutions), 370
16.9.1 Alternatives to In Vivo Parenteral Tests, 371
16.10 Phototoxicity, 371
16.10.1 Theory and Mechanisms, 371
16.10.2 Factors Influencing Phototoxicity/Photosensitization, 372
16.10.3 Predictive Tests for Phototoxicity, 373
16.10.4 3T3 In Vitro Test, 373
16.10.5 Rabbit Phototoxicity Test, 373
16.10.6 Guinea Pig, 374
16.10.7 Pyrogenicity, 376
16.11 Hemocompatibility, 377
References, 378

17 Pharmacokinetics and Toxicokinetics in Drug Safety Evaluation 381
17.1 Introduction, 381
17.2 Regulations, 382
17.3 Principles, 382
17.3.1 Preliminary Work, 382
17.3.2 Absorption, 384
17.3.3 Distribution, 388
17.3.4 Metabolism/Biotransformation, 389
17.3.5 Excretion, 394
17.4 Pharmacokinetics, 395
17.5 Laboratory Methods, 395
17.5.1 Analytical Methods, 395
17.6 Sampling Methods and Intervals, 397
17.6.1 Blood, 397
17.6.2 Excreta, 398
17.6.3 Bile, 398
17.6.4 Expired Air, 398
17.6.5 Milk, 398
17.7 Study Types, 400
17.7.1 Whole-Body Autoradiography, 401
17.7.2 Mass Balance Studies, 402
17.8 Analysis of Data, 402
17.8.1 Use of Data from Metabolism and Pharmacokinetic Studies, 404
17.9 Physiologically Based Pharmacokinetic (PBPK) Modeling, 404
17.10 Points to Consider, 405
17.11 Biologically Derived Materials, 406
17.11.1 Immunoassay Methods, 407
17.12 Points to Consider, 410
References, 410

18 Safety Pharmacology

18.1 Regulatory Requirements, 414
18.2 Study Designs and Principles, 415
18.3 Organ System-Specific Tests, 416
18.3.1 General Considerations in Selection and Design of Safety Pharmacology Studies, 416
18.3.2 Studies on Metabolites, Isomers, and Finished Products, 416
18.4 Cardiovascular, 416
18.4.1 Hemodynamics, ECG, and Respiration in Anesthetized Dogs or Primates, 417
18.4.2 Cardiac Conduction Studies, 417
18.4.3 Conscious Dog, Primate, or Minipig Telemetry Studies, 417
18.4.4 Six-Lead ECG Measurement in the Conscious Dog and Minipig, 417
18.4.5 Systems for Recording Cardiac Action Potentials, 418
18.4.6 Special Case (and Concern): QT Prolongation, 418
18.4.7 Some Specific Techniques Which Can Be Employed, 419
18.4.8 Relevance of hERG to QT Prolongation, 419
18.5 Central Nervous System, 419
18.5.1 Isolated Tissue Assays, 420
18.5.2 Electrophysiology Methods, 421
18.5.3 CNS Function: Electroencephalography, 421
18.5.4 Neurochemical and Biochemical Assays, 421
18.6 Respiratory/Pulmonary System, 422
18.6.1 Design of Respiratory Function Safety Studies, 425
18.6.2 Capnography, 426
18.7 Secondary Organ System, 427
  18.7.1 Gastric Emptying Rate and Gastric pH Changes: A New Model, 427
18.8 Renal Function Tests, 428
18.9 Summary, 428
References, 428

19 Special Concerns for the Preclinical Evaluation of Biotechnology Products 433
19.1 Regulation, 436
19.2 Preclinical Safety Assessment, 437
19.3 Recombinant DNA Technology, 439
  19.3.1 General Safety Issues, 440
  19.3.2 Specific Toxicological Concerns, 440
19.4 Immunogenicity/Allergenicity, 440
19.5 Monoclonal Antibody Technology, 441
  19.5.1 Toxicological Concerns with Monoclonal Antibodies, 442
19.6 Bioprocess Technology, 446
19.7 Gene Therapy Products, 446
  19.7.1 Vectors, 447
  19.7.2 Studies to Support the First Dose in Man, 447
  19.7.3 Distribution of the Gene and Gene Product, 447
  19.7.4 Studies to Support Multiple Doses in Humans, 447
  19.7.5 Unnecessary Studies, 448
  19.7.6 Ex Vivo Procedures, 448
  19.7.7 Change of Gene or Vector, 448
  19.7.8 Change of Route, 448
  19.7.9 Insertional Mutagenesis, 448
19.8 Vaccines, 449
  19.8.1 Approaches to Vaccination, 449
  19.8.2 Genetic Engineering and Vaccine Development, 450
19.9 Special Challenges, 452
  19.9.1 Purity and Homology, 453
  19.9.2 Immunogenicity, 453
19.10 Planning a Safety Evaluation Program, 454
  19.10.1 The Producing System, 454
  19.10.2 The Process, 454
  19.10.3 The Product, 455
  19.10.4 Biology of Bioengineered Products, 455
  19.10.5 Animal Models, 455
  19.10.6 Study Design, 457
  19.10.7 Frequency and Route of Administration, 458
  19.10.8 Duration, 458
  19.10.9 Special Toxicity Testing, 458
  19.10.10 Program Design Considerations, 458
19.11 Challenges: Biosimilars, 458
References, 459

20 Safety Assessment of Inhalant Drugs and Dermal Route Drugs 461
20.1 Inhaled Therapeutics, 461
20.2 The Pulmonary System, 461
20.3 Penetration and Absorption of Inhaled Gases and Vapors, 462
20.4 Deposition of Inhaled Aerosols, 463
20.5 Absorption and Clearance of Inhaled Aerosols, 464
20.6 Pharmacokinetics and Pharmacodynamics of Inhaled Aerosols, 464
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.7</td>
<td>Methods for Safety Assessment of Inhaled Therapeutics</td>
<td>465</td>
</tr>
<tr>
<td>20.8</td>
<td>Parameters of Toxicity Evaluation</td>
<td>467</td>
</tr>
<tr>
<td>20.8.1</td>
<td>The Inhaled “Dose”</td>
<td>467</td>
</tr>
<tr>
<td>20.8.2</td>
<td>The Dose–Response Relationship</td>
<td>468</td>
</tr>
<tr>
<td>20.8.3</td>
<td>Exposure Concentration versus Response</td>
<td>469</td>
</tr>
<tr>
<td>20.8.4</td>
<td>Product of Concentration and Duration ((Ct)) versus Responses</td>
<td>469</td>
</tr>
<tr>
<td>20.8.5</td>
<td>Units for Exposure Concentration</td>
<td>469</td>
</tr>
<tr>
<td>20.9</td>
<td>Inhalation Exposure Techniques</td>
<td>470</td>
</tr>
<tr>
<td>20.10</td>
<td>The Utility of Toxicity Data</td>
<td>473</td>
</tr>
<tr>
<td>20.11</td>
<td>Formulation and Potential Mucosal Damage</td>
<td>473</td>
</tr>
<tr>
<td>20.11.1</td>
<td>Methods to Assess Irritancy and Damage</td>
<td>473</td>
</tr>
<tr>
<td>20.12</td>
<td>Therapeutic Drug Delivery by the Dermal Route</td>
<td>474</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>476</td>
</tr>
<tr>
<td>21</td>
<td>Special Case Products: Imaging Agents</td>
<td>483</td>
</tr>
<tr>
<td>21.1</td>
<td>Introduction</td>
<td>483</td>
</tr>
<tr>
<td>21.2</td>
<td>Imaging Agents</td>
<td>483</td>
</tr>
<tr>
<td>21.2.1</td>
<td>Contrast Agents</td>
<td>484</td>
</tr>
<tr>
<td>21.2.2</td>
<td>Diagnostic Radiopharmaceuticals</td>
<td>484</td>
</tr>
<tr>
<td>21.2.3</td>
<td>Medical Imaging Agent Characteristics Relevant to Safety</td>
<td>485</td>
</tr>
<tr>
<td>21.2.4</td>
<td>Performance of Nonclinical Safety Assessments</td>
<td>485</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>487</td>
</tr>
<tr>
<td>22</td>
<td>Special Case Products: Drugs for Treatment of Cancer</td>
<td>489</td>
</tr>
<tr>
<td>22.1</td>
<td>Introduction</td>
<td>489</td>
</tr>
<tr>
<td>22.1.1</td>
<td>Dose Conversions: Perspective</td>
<td>493</td>
</tr>
<tr>
<td>22.1.2</td>
<td>The Use of the (mg/m^2) Dose Unit</td>
<td>493</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>493</td>
</tr>
<tr>
<td>23</td>
<td>Pediatric Product Safety Assessment (2006 Guidance, Including Juvenile Toxicology)</td>
<td>495</td>
</tr>
<tr>
<td>23.1</td>
<td>Introduction</td>
<td>495</td>
</tr>
<tr>
<td>23.1.1</td>
<td>Scope of Nonclinical Safety Evaluation</td>
<td>497</td>
</tr>
<tr>
<td>23.1.2</td>
<td>Timing of Juvenile Animal Studies in Relation to Clinical Testing</td>
<td>497</td>
</tr>
<tr>
<td>23.2</td>
<td>Issues to Consider Regarding Juvenile Animal Studies</td>
<td>498</td>
</tr>
<tr>
<td>23.2.1</td>
<td>Developmental Stage of Intended Population</td>
<td>498</td>
</tr>
<tr>
<td>23.2.2</td>
<td>Evaluating Data to Determine When Juvenile Animal Studies Should Be Used</td>
<td>498</td>
</tr>
<tr>
<td>23.2.3</td>
<td>Considering Developmental Windows When Determining Duration of Clinical Use</td>
<td>498</td>
</tr>
<tr>
<td>23.2.4</td>
<td>Timing of Exposure</td>
<td>498</td>
</tr>
<tr>
<td>23.2.5</td>
<td>Selection of Study Models</td>
<td>499</td>
</tr>
<tr>
<td>23.3</td>
<td>General Considerations in Designing Toxicity Studies in Juvenile Animals</td>
<td>499</td>
</tr>
<tr>
<td>23.4</td>
<td>Study Designs and Considerations</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>501</td>
</tr>
<tr>
<td>24</td>
<td>Use of Imaging, Imaging Agents, and Radiopharmaceuticals in Nonclinical Toxicology</td>
<td>503</td>
</tr>
<tr>
<td>24.1</td>
<td>Introduction</td>
<td>503</td>
</tr>
<tr>
<td>24.1.1</td>
<td>Multimodality Imaging Techniques</td>
<td>504</td>
</tr>
<tr>
<td>24.1.2</td>
<td>Dynamic Molecular Imaging Techniques</td>
<td>504</td>
</tr>
</tbody>
</table>
CONTENTS

24.2 X-ray, 505
   24.2.1 Angiography, 505
24.3 Positron Emission Tomography (PET), 505
24.4 Single-photon Emission Computed Tomography (SPECT), 505
24.5 Computed Tomography (CT), 506
24.6 Magnetic Resonance Imaging (MRI), 506
24.7 Optical Imaging, 507
24.8 Ultrasound, 508
   24.8.1 Echocardiography, 508
24.9 Nanoparticle Contrast Agents, 509
24.10 Radiopharmaceuticals, 509
24.11 Applications of Preclinical Imaging in Laboratory Animals, 509
   24.11.1 Molecular Imaging as an ADME Platform in Drug Screen, 509
   24.11.2 Preclinical Imaging in Oncology, 510
   24.11.3 Preclinical Imaging of CNS Disease, 514
   24.11.4 Preclinical Imaging of Autoimmune Disease, 514
   24.11.5 Imaging Animal Model of Infectious Disease, 515
   24.11.6 Preclinical Imaging of Cardiac Disease, 515
24.12 Nonclinical Safety Assessment for Imaging Agents, 515
24.13 Radiopharmaceuticals, 517
24.14 Nonclinical Late Radiation Toxicity Studies, 519
   24.14.1 Study Goals, 519
24.15 Study Design, 519
   24.15.1 Good Laboratory Practices, 519
   24.15.2 Species Selection, 519
   24.15.3 Timing of Study, 519
   24.15.4 General Study Design, 519
   24.15.5 Dose Levels, 520
   24.15.6 Clinical Pathology, 520
   24.15.7 Necropsy and Histopathology, 520
References, 520

25 Occupational Toxicology in the Pharmaceutical Industry 523
   25.1 Introduction, 523
   25.2 Occupational Toxicology versus Drug Safety Evaluation, 523
   25.3 Regulatory Pressures in the United States and the European Community, 525
   25.4 Organizational Structure, 526
   25.5 Activities, 527
      25.5.1 Data Evaluation and Dissemination, 527
      25.5.2 Data Development, 528
      25.5.3 Occupational Exposure Limits (OELs), 531
      25.5.4 Hazard Assessment, 531
      25.5.5 Employee Training, 532
   25.6 Conclusion, 534
References, 534

26 Strategy and Phasing for Nonclinical Drug Safety Evaluation in the Discovery and Development of Pharmaceuticals 537
   26.1 Introduction, 537
   26.2 Regulatory Requirements, 539
   26.3 Essential Elements of Project Management, 542
   26.4 Screens: Their Use and Interpretation in Safety Assessment, 544
      26.4.1 Characteristics of Screens, 545
26.5 Strategy and Phasing, 546
26.6 Critical Considerations, 550
26.7 Special Cases in Safety Assessment, 551
26.8 Summary, 551
References, 551

27 The Application of *In Vitro* Techniques in Drug Safety Assessment 553
27.1 Introduction, 553
27.2 *In Vitro* Testing in Pharmaceutical Safety Assessment, 555
27.3 Defining Testing Objective, 558
    27.3.1 Objectives behind Data Generation and Utilization, 558
27.4 Test Systems: Characteristics, Development, and Selection, 558
27.5 *In Vitro* Models, 559
27.6 Lethality, 560
    27.6.1 Ocular Irritation, 564
    27.6.2 Dermal Irritation, 564
    27.6.3 Irritation of Parenterally Administered Pharmaceuticals, 565
    27.6.4 Sensitization and Photosensitization, 566
    27.6.5 Phototoxicity and Photosensitization, 567
    27.6.6 Developmental Toxicity, 568
    27.6.7 Target Organ Toxicity Models, 568
27.7 *In Silico* Methods, 572
27.8 The Final Frontier and Barrier: Regulatory Acceptance, 573
27.9 Summary, 573
References, 575
Further Reading, 581

28 Evaluation of Human Tolerance and Safety in Clinical Trials: Phase I and Beyond 583
28.1 The Pharmaceutical Clinical Development Process and Safety, 583
    28.1.1 Pharmacokinetics, 589
    28.1.2 Safety of Clinical Trial Subjects, 591
28.2 Limitations on/of Clinical Trials, 598
28.3 The Clinical Trial Process, 598
    28.3.1 Development of an Application Unrelated to Original Approved Use, 601
28.4 Institutional Review Boards (IRBS)/Ethics Committees in the Clinical Trial Process, 602
    28.4.1 Legal Authority and Responsibilities for IRBs, 602
    28.4.2 Duties of IRBs, 603
    28.4.3 Informed Consent, 603
28.5 Drug Formulations and Excipients, 604
    28.5.1 Route of Administration, 605
28.6 Phase I Designs, 605
    28.6.1 First Administration: Single Dose Escalating (SDE), 606
    28.6.2 First Administration in Humans: Multiple Dose Escalating (MDE), 608
28.7 Clinical Trial Safety Indicators, 609
    28.7.1 Overall Approach to Assessing Safety, 609
    28.7.2 Precautions, 610
    28.7.3 Clinical Chemistry, 613
    28.7.4 Urinalysis, 614
    28.7.5 Urine Screens, 614
28.7.6 Identifying New Diagnostic Laboratory Tests, 614
28.7.7 Ophthalmological Examination, 614
28.7.8 Dermatological Examinations, 614
28.7.9 Cardiovascular Safety, 615
28.7.10 Deaths in Clinical Trials, 615
28.7.11 Behavioral Rating Scales, Performance, Personality, and Disability Tests, 616
28.7.12 Adult Behavioral Rating Scales, 616
28.7.13 Pediatric Behavioral Rating and Diagnostic Scales, 618
28.7.14 Psychometric and Performance Tests, 619
28.7.15 Personality Tests, 621

28.8 Assessment of Unwanted Drug Effects, 621
28.8.1 Separation of Adverse Reactions from Placebo Reactions, 621

References, 626

29 Postmarketing Safety Evaluation: Monitoring, Assessing, and Reporting of Adverse Drug Responses (ADRs) 629
29.1 Causes of Safety Withdrawals, 637
29.2 Regulatory Requirements, 638
29.2.1 The 15-Day Report versus the US Periodic Report, 639
29.3 Management of ADR and ADE Data, 641
29.3.1 Sources of Data, 641
29.3.2 Clinical Trials, 641
29.3.3 Postmarketing Surveillance Studies, 641
29.3.4 Spontaneous Reports, 641
29.3.5 Literature, 642
29.3.6 Searching for ADRs in the Literature, 642
29.3.7 Information Required for Reports, 642
29.3.8 Adverse Drug Reaction Forms and Form Design, 642
29.3.9 Computerization of Drug Safety Data: Data Collection and Input, 644
29.3.10 Medical and Drug Terminology, 644
29.3.11 Dictionaries, 645
29.3.12 Medical Term Coding Dictionaries, 645
29.3.13 Medical Dictionary for Regulatory Activities, 645
29.3.14 Periodic Reports, 646
29.4 Causality Assessment, 647
29.4.1 Aims of Causality Assessment, 647
29.5 Courses of Corrective Action, 647
29.6 Legal Consequences of Safety Withdrawal, 648
29.6.1 FDA Tools for Risk Management, 648
29.6.2 Tier 1: Mandatory Studies, 649
29.6.3 Tier 2: Labeling and Assessment, 649
29.6.4 Tier 3: Enhanced Communication, 650
29.6.5 Tier 4: Safe Use Restriction Defined by Provider, 650
29.6.6 Tier 5: Safe Use Restriction Defined by Patient, 651

References, 651

30 Statistics in Pharmaceutical Safety Assessment 653
30.1 Introduction, 653
30.1.1 Bias and Chance, 655
30.1.2 Hypothesis Testing and Probability (p) Values, 655
30.1.3 Multiple Comparisons, 656
30.1.4 Estimating the Size of the Effect, 656
30.1.5 Functions of Statistics, 657
30.1.6 Descriptive Statistics, 658

30.2 Experimental Design, 659
30.2.1 Choice of Species and Strain, 659
30.2.2 Sampling, 659
30.2.3 Dose Levels, 660
30.2.4 Number of Animals, 660
30.2.5 Duration of the Study, 660
30.2.6 Stratification, 661
30.2.7 Randomization, 661
30.2.8 Adequacy of Control Group, 661

30.3 Data Recording, 664

30.4 Generalized Methodology Selection, 665

30.5 Statistical Analysis: General Considerations, 665
30.5.1 Variables to Be Analyzed, 665
30.5.2 Combination of Observations
   (Such as Pathological Conditions), 667
30.5.3 Taking Severity into Account, 668
30.5.4 Using Simple Methods Which Avoid Complex Assumptions, 668
30.5.5 Using All the Data, 668
30.5.6 Combining, Pooling, and Stratification, 668
30.5.7 Trend Analysis, Low-Dose Extrapolation, and NOEL Estimation, 669
30.5.8 Need for Age Adjustment, 671
30.5.9 Need to Take Context of Observation into Account, 672
30.5.10 Experimental and Observational Units, 672
30.5.11 Missing Data, 672
30.5.12 Use of Historical Control Data, 673
30.5.13 Methods for Data Examination and Preparation, 673
30.5.14 Scattergram, 673
30.5.15 Bartlett’s Test for Homogeneity of Variance, 675
30.5.16 Statistical Goodness-of-Fit Tests, 676
30.5.17 Randomization, 677
30.5.18 Transformations, 677
30.5.19 Exploratory Data Analysis, 678

30.6 Hypothesis Testing of Categorical and Ranked Data, 679
30.6.1 Fisher’s Exact Test, 679
30.6.2 2×2 Chi-Square, 680
30.6.3 R×C Chi-Square, 680
30.6.4 Wilcoxon Rank-Sum Test, 681
30.6.5 Distribution-Free Multiple Comparison, 682
30.6.6 Mann–Whitney U Test, 682
30.6.7 Kruskal–Wallis Nonparametric ANOVA, 683
30.6.8 Log-Rank Test, 683

30.7 Hypothesis Testing: Univariate Parametric Tests, 684
30.7.1 Student’s t-Test (Unpaired t-Test), 685
30.7.2 Cochran t-Test, 685
30.7.3 F-Test, 686
30.7.4 Analysis of Variance (ANOVA), 686
30.7.5 Post Hoc Tests, 687
30.7.6 Duncan’s Multiple Range Test, 687
30.7.7 Groups with Equal Number of Data \((N_1=N_2)\), 687
30.7.8 Groups with Unequal Number of Data \((N_1\neq N_2)\), 688
30.7.9 Scheffe’s Multiple Comparisons, 688
30.7.10 Dunnett’s t-Test, 688
30.7.11 Williams’ t-Test, 689
30.7.12 Analysis of Covariance, 689
30.7.13 Modeling, 690
30.7.14 Linear Regression, 691
30.7.15 Probit/Log Transforms and Regression, 691
30.7.16 Nonlinear Regression, 692
30.7.17 Correlation Coefficient, 693
30.7.18 Kendall’s Coefficient of Rank Correlation, 694
30.7.19 Trend Analysis, 694
30.8 Methods for the Reduction of Dimensionality, 694
30.8.1 Classification, 695
30.8.2 Statistical Graphics, 696
30.8.3 Multidimensional and Nonmetric Scaling, 697
30.8.4 Cluster Analysis, 699
30.8.5 Fourier or Time Analysis, 699
30.8.6 Life Tables, 700
30.9 Meta-Analysis, 701
30.9.1 Selection of the Studies to Be Analyzed, 701
30.9.2 Pooled (Quantitative) Analysis, 701
30.9.3 Methodological (Qualitative) Analysis, 702
30.10 Bayesian Inference, 702
30.10.1 Bayes’ Theorem and Evaluation of Safety Assessment Studies, 702
30.10.2 Bayes’ Theorem and Individual Animal Evaluation, 703
30.11 Data Analysis Applications in Safety Assessment Studies, 704
30.11.1 Body and Organ Weights, 705
30.11.2 Clinical Chemistry, 706
30.11.3 Hematology, 706
30.11.4 Histopathological Lesion Incidence, 706
30.11.5 Carcinogenesis, 707

References, 708

31 Combination Products: Drugs and Devices 711
31.1 Combination Products, 711
31.1.1 Historical Background, 711
31.1.2 Future Trends, 712

References, 720

32 Qualification of Impurities, Degradants, Residual Solvents, Metals, and Leachables in Pharmaceuticals 721
32.1 Impurities, 721
32.2 Residual Solvents, 726
32.3 Extractables and Leachables, 727
32.4 Residual Metals and Elements, 728

References, 730

33 Tissue, Cell, and Gene Therapy 731
33.1 Safety Assessment of Cell Therapy (CT) Products, 732
33.1.1 Recommendations for General Preclinical Program Design, 732
33.1.2 Model Species Selection, 732
33.1.3 Selection of Animal Models of Disease/Injury, 732
CONTENTS

33.1.4 Information Describing Limitations of Potential Animal Model(s), 733
33.1.5 Information Supporting the Choice of Animal Model(s), 733
33.1.6 Proof-of-Concept (POC) Studies, 733
33.1.7 Toxicology Studies, 734
33.1.8 Product Delivery Considerations, 735
33.1.9 Study Designs, 736
33.1.10 CT Products with Implantable Scaffolds, 738

33.2 Nonclinical Safety Assessment of Gene Therapy Products (GTPS), 738
  33.2.1 CBER, 738
  33.2.2 NIH, 738
  33.2.3 Study Designs, 739
  33.2.4 Ex Vivo Genetically Modified Cells, 740
  33.2.5 Biodistribution Considerations, 740

33.3 Definitions, 741

References, 742

Appendix A: Selected Regulatory and Toxicological Acronyms 743
Appendix B: Definition of Terms and Lexicon of “Clinical” Observations in Nonclinical (Animal) Studies 745
Appendix C: Notable Regulatory Internet Addresses 749
Appendix D: Glossary of Terms Used in the Clinical Evaluation of Therapeutic Agents 755
Appendix E: Common Vehicles for the Nonclinical Evaluation of Therapeutic Agents 759
Appendix F: Global Directory of Contract Pharmaceutical Toxicology Labs 857

INDEX 879