
Contents

Preface.....	xix
Acknowledgments.....	xxi
Author	xxiii

SECTION I Basic Concepts in Toxicology Testing

Chapter 1 Introduction to Principles of Toxicology.....	3
1.1 Introduction	3
1.2 Types of Toxicology.....	3
1.2.1 General Toxicology	3
1.2.2 Mechanistic Toxicology	3
1.2.3 Regulatory Toxicology	4
1.2.4 Descriptive Toxicology and Clinical Toxicology	4
1.3 Common Terms and Nomenclature.....	5
1.4 Applications of Toxicology	5
1.4.1 Research	5
1.4.1.1 Academic Applications.....	5
1.4.1.2 Industrial Applications.....	5
1.4.2 Regulatory Toxicology	5
1.4.3 Forensic Toxicology	6
1.4.4 Clinical Toxicology	6
1.5 Classification of Toxic Agents	6
1.5.1 Classification According to Use	7
1.5.1.1 Pesticides.....	7
1.5.1.2 Food and Industrial Additives	7
1.5.1.3 Therapeutic Drugs	8
1.6 Sources of Toxins.....	8
1.6.1 Botanical.....	8
1.6.2 Environmental	8
Suggested Readings	8
Review Articles	9
Chapter 2 Effects of Chemicals.....	11
2.1 Toxicological Effects.....	11
2.1.1 General Classification	11
2.1.2 Chemical Allergies	11
2.1.3 Idiosyncratic Reactions	13

2.1.4	Immediate versus Delayed Hypersensitivity	14
2.1.5	Reversible versus Irreversible Effects.....	14
2.1.6	Local versus Systemic Effects	14
2.1.7	Mutagenic and Carcinogenic Effects.....	14
2.2	Biochemical Properties	15
2.2.1	Chemical Structure.....	15
2.2.2	Mechanism of Action.....	15
2.3	Exposure.....	15
2.3.1	Route	15
2.3.1.1	Oral Administration	15
2.3.1.2	Intranasal Administration.....	17
2.3.1.3	Inhalation	17
2.3.1.4	Dermal and Parenteral Routes.....	17
2.3.2	Duration and Frequency.....	18
2.3.2.1	Acute Exposure.....	18
2.3.2.2	Chronic Exposure	19
2.3.3	Accumulation	19
2.3.3.1	According to Physiological Compartment.....	19
2.3.3.2	According to Chemical Structure.....	20
2.4	Chemical Interactions	20
2.4.1	Potentiation.....	20
2.4.2	Additive Effects.....	20
2.4.3	Synergistic Effects.....	20
2.4.4	Antagonistic Effects	21
2.5	Dose–Response Relationship.....	21
2.5.1	General Assumptions	21
2.5.1.1	Graded Dose–Response.....	21
2.5.1.2	Quantal Dose–Response.....	22
2.5.2	Concentration	23
2.5.3	Criteria for Measurement.....	23
	Suggested Readings	24
	Review Articles	24
	Chapter 3 Toxicokinetics	27
3.1	Introduction	27
3.1.1	Relationship to Pharmacokinetics.....	27
3.1.2	One-Compartment Model	27
3.1.3	Two-Compartment Model	28
3.1.4	Applications to Toxicology Testing	28
3.2	Absorption	28
3.2.1	Ionic and Non-Ionic Principles	28
3.2.2	Henderson-Hasselbach Equation	30
3.2.3	Absorption in Nasal and Respiratory Mucosa	32
3.2.3.1	Nasal Mucosa.....	32
3.2.3.2	Respiratory Mucosa	32

5.1.2	Predictive Toxicology and Extrapolation to Human Toxicity.....	57
5.2	Animal Welfare and U.S. Animal Welfare Act.....	57
5.3	Chemicals	59
5.3.1	Selection of Chemicals	59
5.3.1.1	Solubility.....	59
5.3.1.2	Vehicle.....	5 9
5.3.2	Route of Administration.....	60
5.4	Species Differentiation.....	60
5.4.1	Selection of Appropriate Animal Species.....	60
5.4.2	Cost Effectiveness.....	63
5.4.3	Animal Housing	64
5.4.4	Diet	64
5.4.5	Institutional Animal Care and Use Committees (IACUCs).....	66
5.5	Methodologies.....	67
5.5.1	Routes of Exposure, Duration, and Frequency of Dosing	67
5.5.2	Toxicity Indicators	67
	Suggested Readings.....	68
	Review Articles.....	69

SECTION II Toxicology Testing In Vivo

Chapter 6	Acute Toxicology Testing	73
6.1	Objectives of Acute Toxicology Tests	73
6.2	LD ₅₀ and Acute Toxicology Tests.....	73
6.3	Organization of Studies.....	75
6.4	Range-Finding Tests.....	75
6.4.1	Up-and-Down Procedure (UDP).....	76
6.4.2	Fixed Dose Procedure (FDP).....	77
6.4.3	Acute Toxic Class Method (ATCM).....	77
6.5	Classical LD,,	78
6.5.1	Oral LD ₅₀	78
6.5.2	Dermal LD,,	7.9
6.5.3	Inhalation LD ₅₀	80
6.6	Other Considerations with LD,, Determinations	80
6.6.1	Route of Administration.....	81
6.6.2	Duration.....	82
6.6.3	General Appearance of Animals.....	8 2
6.6.4	Specimen Collection and Gross Pathology	82
6.6.5	Biological Variation.....	83
6.6.6	Determination of Acute Lethality	83
6.7	Applications of LD,, Studies.....	86
	Suggested Readings.....	86
	Review Articles.....	87

Chapter 7	Subchronic and Chronic Toxicology Testing	89
7.1	Introduction	89
7.2	Types of Subchronic and Chronic Toxicity Tests.....	89
7.2.1	Objectives and Definitions.....	89
7.2.2	Factors Associated with Chronic Toxicity.....	90
7.2.2.1	Frequency of Exposure.....	90
7.2.2.2	Accumulation and Distribution	90
7.2.2.3	Influence of Chemical Structure.....	91
7.2.3	Objectives of Chronic Toxicity Studies	91
7.3	Experimental Design.....	92
7.3.1	Selection of Dosage Levels	92
7.3.2	Selection of Animals	92
7.3.3	Monitoring Daily Criteria of Chronic Toxicology Studies	94
7.3.4	Duration of Studies	96
7.3.5	Recovery Experiments	96
7.3.6	Analysis of Results	97
	Suggested Readings	99
	Review Articles.....	99
Chapter 8	Acute Dermal and Ocular Toxicity Testing.....	101
8.1	Introduction	101
8.2	Acute Dermal Toxicity Tests	101
8.2.1	Description	101
8.2.2	Primary Irritation.....	101
8.2.2.1	Study Design and Procedures.....	103
8.2.3	Skin Sensitization.....	103
8.2.3.1	Study Design and Procedures.....	104
8.2.4	Photoallergic and Phototoxic Reactions	105
8.2.4.1	Study Design and Procedures.....	107
8.3	Acute Ocular Toxicity Tests	107
8.3.1	Eye Irritation and Corrosion Testing.....	107
8.3.2	Objections to and Limitations of Eye Irritation and Corrosion Testing	108
8.4	Conclusions	108
	Suggested Readings	109
	Review Articles.....	110
Chapter 9	Toxicity Testing for Fertility and Reproduction.....	111
9.1	Introduction	111
9.2	History and Development of Teratogenicity Testing.....	112
9.3	Brief Description of Maternal–Fetal Physiology	112
9.3.1	Fetal–Prenatal Development	112
9.3.2	First Trimester	113
9.3.3	Second and Third Trimesters	114

9.4	Mechanisms of Developmental Toxicity	114
9.4.1	Susceptibility	114
9.4.2	Dose–Response and Threshold	114
9.5	Teratogenicity.....	115
9.5.1	Teratogenic Agents.....	115
9.5.2	Factors Associated with Teratogenicity.....	116
9.5.3	Animal Tests for Teratogenicity	117
9.5.3.1	Standard Protocols	118
9.6	Embryotoxicity.....	119
9.6.1	Whole Embryo Culture.....	119
9.6.1.1	Pre-implantation Techniques	119
9.6.1.2	Post-Implantation Embryo Culture	120
9.6.1.3	Organ Cultures.....	121
9.6.1.4	International Guidelines	121
9.7	Male Reproductive Toxicology.....	122
9.7.1	Spermatogenesis.....	122
9.7.2	Reproductive Tests	123
9.8	Female Reproductive Toxicology	125
9.8.1	Oogenesis	125
9.8.2	Reproductive Tests	127
	Suggested Readings.....	128
	Review Articles.....	129

Chapter 10	Carcinogenicity and Mutagenicity Testing <i>In Vivo</i>	131
10.1	Introduction and Definitions.....	131
10.2	Multistage Carcinogenesis	132
10.2.1	Tumor Initiation	132
10.2.2	Tumor Promotion	132
10.2.3	Tumor Progression	133
10.3	Carcinogenic and Genotoxic Agents	133
10.4	Carcinogenicity Testing <i>In Vivo</i>	135
10.4.1	Principles of <i>In Vivo</i> Carcinogenicity Testing.....	135
10.4.2	<i>In Vivo</i> Genotoxicity and Cytogenetic Tests	137
10.4.3	Conclusions	137
10.5	Bioassay Protocols for <i>In Vivo</i> Carcinogenicity Testing.....	138
10.5.1	Study Duration	138
10.5.2	Indicators for Detection of Carcinogens	138
10.5.3	Selection of Animal Species and Numbers	139
10.5.4	Exposure Levels	139
10.5.5	Sources of Variability	140
10.5.6	Carcinogenicity Bioassays	140
10.5.6.1	Short-Term Test Batteries.....	140
10.5.6.2	Tier Testing	141
10.5.6.3	Decision Point Analysis.....	141
10.5.6.4	Rodent Liver Altered Foci Induction	142

10.6 Mutagenicity Testing <i>In Vivo</i>	142
Suggested Readings	142
Review Articles.....	143

SECTION III Toxicology Testing *In Vitro*

Chapter 11 Introduction to <i>In vitro</i> Toxicology Testing	147
11.1 History of <i>In Vitro</i> Methods.....	147
11.2 <i>In vitro</i> Systems	148
11.3 History of Cell Culture	148
11.3.1 Tissue Explants	148
11.3.2 Recent Developments.....	149
Suggested Readings	150
Review Articles.....	150
Chapter 12 Cell Culture Methodology	151
12.1 Cell Culture Laboratory	151
12.1.1 Equipment	151
12.2 Cultured Cells	152
12.2.1 Primary Cultures	152
12.2.2 Finite Continuous Cell Lines.....	153
12.2.3 Immortal Continuous Cell Lines	154
12.2.4 Stem Cell Lines.....	155
12.2.4.1 Influence of Culture Environment on ES cells	156
12.2.4.2 Culture of Human or Mouse ES cells.....	157
12.2.5 Clonal Growth and Maintenance Cultures	158
12.2.6 Criteria for Identification and Monitoring of Cultured Cells	159
12.2.6.1 Karyotypic Analysis.....	159
12.2.6.2 Aging in Culture	159
12.2.6.3 Anchorage-Dependent Cultures.....	159
12.2.6.4 Contact Inhibition	160
12.3 Culture Requirements	161
12.3.1 Media Components.....	161
12.3.2 Temperature.....	162
12.3.3 pH	162
12.3.4 Carbon Dioxide Tension	162
12.3.5 Buffering	163
12.3.6 Osmolality	163
12.3.7 Water Requirement.....	164
12.3.8 Glassware Requirement	164
12.3.9 Serum-Free Medium	164
12.4 Procedures	165
12.4.1 Static and Perfusion Cell Systems.....	165

12.4.2 Dispersion of Tissues	166
12.4.3 Subculturing	166
12.4.4 Measurement of Growth and Viability	168
12.4.5 Cryopreservation	169
12.4.6 Cell Identification.....	171
12.4.7 Large Scale Bioprocesses	171
12.4.8 Sources of Cell Lines.....	172
Suggested Readings.....	172
Review Articles.....	172
Chapter 13 Cell Culture Methods for Acute Toxicology Testing	175
13.1 Introduction	175
13.1.1 Testing Principles	175
13.1.2 Criteria.....	176
13.1.3 Viability Assays.....	181
13.1.3.1 NRU Assay	181
13.1.3.2 MTT Assay	182
13.1.4 Conclusions	182
13.2 Acute Target Organ Toxicology Testing.....	184
13.2.1 Use of Specialized Primary Cultures	184
13.2.2 Establishment of Primary Cultures of Hepatocytes	185
13.2.2.1 <i>In Situ</i> Liver Perfusion	186
13.2.2.2 Primary Hepatocyte Enrichment	187
13.2.2.3 Hepatocyte Cell Culture	187
13.2.2.4 Functional Markers of Primary Hepatocyte Cultures	187
13.2.3 Establishment of Primary Cultures from Other Organs.....	188
13.2.3.1 Isolation of Renal Cortical Cells.....	188
13.2.3.2 Use of Continuous Renal Cell Lines	189
13.2.3.3 Establishment of Primary Cultures of Lung Cells.....	190
13.2.4 Differential Toxicology Testing	192
13.3 Acute Local Toxicology Testing.....	192
13.3.1 Ocular Toxicology Testing.....	193
13.3.1.1 HET-CAM Test.....	194
13.3.1.2 Bovine Corneal Opacity and Permeability (BCOP) Assay.....	195
13.3.1.3 Isolated Chicken Eye (ICE) Test.....	196
13.3.1.4 Isolated Rabbit Eye (IRE) Test	197
13.3.1.5 EpiOcular™ Model	197
13.3.2 Dermal Toxicology Testing.....	198
13.3.2.1 Murine Local Lymph Node Assay (LLNA).....	198
13.3.2.2 Corrositex™ Assay	199
13.3.2.3 EpiDerm™, Episkin™, and TER	199
13.4 Summary	200
Suggested Readings.....	200
Review Articles.....	201

Chapter 14 Toxicokinetic Studies In Vitro.....	203
14.1 Introduction.....	203
14.2 Metabolic Studies in Cell Culture	203
14.2.1 Biotransformation in Cell Culture	204
14.2.1.1 Enzymatic Metabolism	204
14.2.1.2 Use of S9 Mixture	205
14.2.1.3 Preparation of S9 Fractions	206
14.2.2 <i>In Vitro</i> Studies of Absorption, Distribution, Metabolism, and Elimination (ADME)	206
Suggested Readings	207
Review Articles.....	208
Chapter 15 Mutagenicity Testing <i>In Vitro</i>	209
15.1 Introduction.....	209
15.2 Bacterial Cell Systems	210
15.2.1 Ames Test.....	211
15.2.1.1 Spot Test	213
15.2.1.2 Standard Ames Test	213
15.3 Mammalian Cell Systems	214
15.3.1 Basis of Mutagenicity Testing with Mammalian Systems.....	214
15.3.2 Cell Lines	215
15.3.2.1 CHO and V79 Cells.....	215
15.3.2.2 Mouse Lymphoma L5178Y Cells	216
15.3.3 Cytogenetic Testing.....	216
15.3.3.1 Chromosome Aberrations	216
15.3.3.2 Sister Chromatid Exchange	217
15.3.3.3 Detection of Micronuclei.....	219
15.3.3.4 Limitations of Short-Term Cytogenetic Testing	219
15.3.4 Unscheduled DNA Synthesis (UDS).....	219
15.3.4.1 Principles of Testing.....	219
15.3.5 Cell Transformation	220
15.3.5.1 Principles of Testing.....	220
15.3.5.2 Focus Transformation Assay	220
15.3.5.3 Syrian Hamster Embryo (SHE) Cell Transformation Assay	221
15.3.5.4 Viral and Chemical Transformation Methods.....	222
15.4 Development of Short-Term Test Strategies.....	222
15.5 Summary	225
Suggested Readings	226
Review Articles.....	227
Chapter 16 Reproductive and Teratogenicity Studies <i>In vitro</i>.....	229
16.1 Introduction	229
16.2 Alternative Methods for Embryotoxicity Testing.....	230

16.2.1 Whole Embryo Culture	231
16.2.2 Cell Culture Methods.....	231
16.2.2.1 Pre-Implantation Techniques	231
16.2.2.2 Continuous Cell Cultures	232
16.2.2.3 Embryonic Stem (ES) Cells	232
16.2.3 Organ Cultures	233
16.3 Validation of Alternative Methods for Reproductive and Teratogenicity Studies	234
16.4 Summary	234
Suggested Readings	235
Review Articles.....	235
 Chapter 17 High Throughput Screening and Microarray Analysis	 237
17.1 High Throughput Screening in Preclinical Drug Testing.....	237
17.1.1 Introduction	237
17.1.2 Procedures	237
17.1.3 Equipment	238
17.1.4 Applications	238
17.1.5 Future Directions in HTS	238
17.2 Microarray Systems	239
17.2.1 Introduction	239
17.2.2 Types of Arrays	239
17.2.3 DNA Microarray Design.....	239
17.2.4 Fabrication.....	240
17.2.5 Detection Technology	241
17.2.6 Applications of cDNA Microarray Technology	241
17.2.6.1 Gene Expression	241
17.2.6.2 Genotype/SNP Detection.....	241
17.2.6.3 Detection of Environmental Agents	242
Suggested Readings	243
Review Articles	243
 Chapter 18 Experimental Design and Statistics	 245
18.1 Establishing Experimental Plan	245
18.1.1 Materials and Supplies.....	245
18.1.2 Methods	245
18.2 Experimental Design.....	247
18.2.1 Medium and Chemical Compatibility	247
18.2.1.1 Micronization	248
18.2.1.2 Solvents	248
18.2.1.3 Sonication	249
18.2.1.4 Paraffin (Mineral) Oil Overlay	249
18.2.2 Calculation of Concentration Range.....	250
18.2.3 Determination of Inhibitory Concentrations.....	250

18.3 Hypothesis Testing and Statistical Applications	252
18.3.1 Development of Hypothesis Test.....	252
18.3.2 Parametric Calculations	252
18.3.2.1 Regression Analysis.....	253
18.3.2.2 Correlation Analysis	254
18.3.2.3 Hypothesis Test for $\beta = 0$	254
18.3.2.4 Analysis of Variance (ANOVA) and F-Test.....	255
18.3.2.5 Student's t-Test	256
18.3.3 Non-Parametric or Goodness-of-Fit Calculations	256
18.3.3.1 Chi-square (χ^2).....	257
18.3.3.2 Wilcoxon Rank Sum.....	257
18.3.3.3 Kruskal-Wallis Non-Parametric ANOVA	257
Suggested Readings.....	258
Review Articles.....	258

Chapter 19 Standardization and Validation of Alternative Methods..... 259

19.1 Introduction.....	259
19.1.1 Development of Alternative Test Methods	259
19.1.2 Standardization of Test Protocols	260
19.2 Validation Process	261
19.2.1 Definitions.....	261
19.2.2 Vital Components of Validation Program.....	261
19.2.2.1 Relevance and Reliability	261
19.2.2.2 Selection of Chemicals.....	262
19.2.2.3 Comparison to <i>In Vivo</i> Data	263
19.2.2.4 Comparison to Other <i>In Vitro</i> Protocols	264
19.3 Current Validation Programs.....	265
19.3.1 History.....	265
19.3.2 Advantages of Organized Multilaboratory Validation Programs.....	266
19.3.3 Organizations Dedicated to Development of Alternative Models.....	267
19.3.4 Organized Programs.....	270
19.4 Summary	270
Suggested Readings.....	271
Review Articles.....	272

Chapter 20 Applications of Alternative Models for Toxicology Testing..... 275

20.1 Introduction.....	275
20.1.1 Promulgation of OECD and FRAME Regulations by the European Union	275
20.1.2 Establishment of ICCVAM, NICEATM, and ECVAM Regulatory Agencies	276

20.1.3	Establishment of International Efforts for Development of Alternative Methods	278
20.1.4	Workshops and Validation Programs	278
20.2	Significance of Alternative Models for Animal Toxicology Testing	279
20.2.1	In <i>Vitro</i> Tests and Significance	279
20.2.2	Functional Classification of Cytotoxicity Based on In <i>Vitro</i> Methodologies.....	280
20.2.2.1	Basal Cell Functions.....	280
20.2.2.2	Origin-Specific Cell Functions.....	281
20.2.2.3	Extracellular Functions.....	281
20.2.3	Cytotoxic Classification Based on In <i>Vitro</i> Methodologies.....	281
20.2.3.1	Basal Cytotoxicity	281
20.2.3.2	Organ-Specific Cytotoxicity	283
20.2.3.3	Organizational Cytotoxicity.....	284
20.2.4	Measurements of Acute and Chronic Toxicity	284
20.2.5	Results and Mechanistic Implications	285
20.3	Extrapolation to Human Toxicity	286
20.3.1	Standardized Test Batteries.....	286
20.3.2	Human Risk Assessment.....	288
20.4	Use of Alternative Methods for Human and Animal Toxicology.....	288
	Suggested Readings.....	289
	Review Articles.....	289
	Index.....	2-9 1