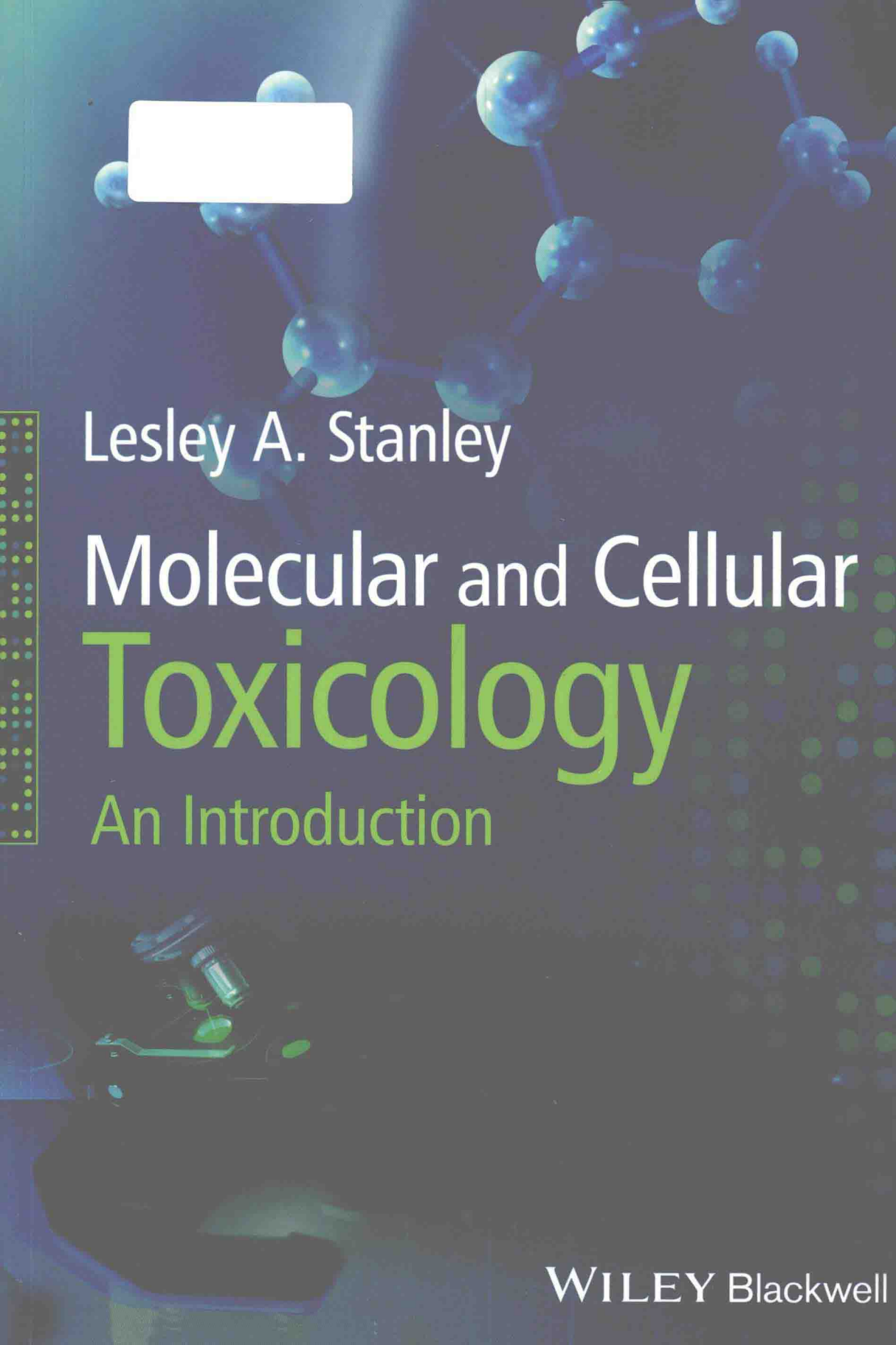

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Molecular and Cellular Toxicology

An Introduction

WILEY Blackwell

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Molecular and Cellular Toxicology

Dedication

Dedicated to the memory of Elizabeth Stanley and Margaret Orr,
two wonderful mothers

Foreword

by Dr Marilyn J. Aardema
Marilyn Aardema Consulting, LLC

Technical advances and bold initiatives like the National Academy of Science's *Toxicology in the 21st Century*, along with socio-political pressures such as the 3Rs (replace, reduce and refine the use of animals in experiments) have led to remarkable changes in the field of toxicology over the past several years. Toxicology is undergoing a major shift towards assessing and understanding damage at the cellular and tissue level. This comprehensive, well-written book focusses on the timely topic of current advances in the field of molecular and cellular toxicology. The book starts with a review of how cells and tissues respond to damage and the consequences of damage that overwhelm normal cellular protective mechanisms. With this background, new technologies for evaluating cellular and tissue damage along with investigating the toxicological outcomes are described. This includes the use of 'omics technologies (transcriptomics (changes in RNA), proteomics (changes in proteins)), metabolomics (changes in products of metabolism; Chapter 3), the use of 3D tissue models to obtain a more biologically relevant assessment of toxicity (Chapters 4, 8) and the use of *in silico* approaches for predicting toxicological effects (Chapter 6). The final Chapter 10 provides a glimpse forward at emerging technologies that are sure to impact the field of molecular and cellular toxicology further in the years ahead. These and the other chapters in the book not only provide essential reading on recent technology developments, but also provide up-to-date information on the drivers behind these advances, and the global efforts towards validation and incorporation of new approaches into the toxicology paradigm.

This book will be invaluable to all those interested in the latest advances in toxicology including postgraduate/graduate life science students interested in toxicology as well as individuals starting out in the field of cosmetics, consumer products, pharmaceutical and testing industries who need knowledge of current approaches in toxicology. I commend the editors and author, Dr Lesley Stanley, on this valuable contribution to the field of Toxicology.

Preface

Over the past 10 years the subject of toxicology has changed dramatically, moving from a discipline which was once firmly wedded to traditional (some might say old-fashioned) methods to one which is keen to embrace the innovative techniques emerging from the developing fields of cell culture and molecular biology. Over the same period our ability to predict outcomes using computer models has also progressed to an astonishing degree.

The availability of novel methods has had a great deal of influence on the development of new approaches in toxicology; another key impetus has been the need to reduce the use of animals in experimentation and testing. This was originally driven by public distaste (even revulsion) for the practice. The scientific approach has been codified in terms of the so-called '3Rs' (Reduction, Refinement and Replacement) and is now enshrined in legislation, particularly the 7th Amendment to the European Union Cosmetics Directive and the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulations.

The 7th Amendment prohibits the marketing within the EU of cosmetics containing ingredients which have been tested on animals. This has made the development of 'alternative' (non-animal and computer-based) methods a key priority, particularly for the cosmetics industry. At the same time, the pharmaceutical industry has been pushed in the same direction by the need for cost-effective ways of screening large numbers of potential drug molecules to identify those with the elusive property of 'druggability' (the rare combination of efficacy and pharmacological properties which allows oral dosing one or a few times per day without accumulation to toxic levels). With these factors in mind, I hope that this book will be useful to individuals at the start of their careers in these industries.

The REACH initiative was first suggested in an EC White Paper released in 2001. A formal legislative proposal was issued in 2003 and the regulations came into force on 1 June 2007. REACH requires companies which manufacture or import chemical substances in quantities greater than 1 tonne to assess their adverse effects in terms of toxicology and consequences of release into the environment. It differs from previous legislation in that it makes the industry responsible for managing the risks associated with the chemicals it uses. Another

important feature of REACH is that it explicitly states that, where possible, animal tests should be replaced by alternatives such as *in vitro* tests or computer predictions.

The aim of this book is to introduce recent developments in the fields of molecular and cellular toxicology to an audience of life scientists at the final year undergraduate and early postgraduate levels. The wide ranging nature of the subject and the rapid rate of progress, however, mean that it cannot be comprehensive; instead, what has been attempted is a broad-brush sketch of the landscape illustrated with examples which highlight the key points and interesting developments.

It will be evident from the wide range of topics mentioned even in this brief preface that, in order to keep the book concise and readable (as well as affordable), a certain amount of background knowledge has had to be assumed. In particular, the reader will need to have a solid foundation in the following:

- Basic biochemistry, molecular and cellular biology
- Conventional laboratory methods using DNA, RNA and protein
- Tissue structure and histology
- Pathological processes and their histopathological consequences

An elementary understanding of the metabolism and toxic effects of chemicals would be helpful but is not essential, since the book aims to cover the relevant processes.

For those who do need to look up some background information on these topics, I recommend the following textbooks:

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I am also grateful to Mrs Roberta Logan and Drs Eian Massey, Robin Whelpton and Gary Hutchison, all of whom provided constructive comments on various versions of the manuscript during its preparation.

This project could not have been completed without the encouragement of many of my friends. In particular, I would like to thank Sarah Young for letting me work in her house while the builders were in mine and Julie McDowell for being my gym buddy.

Finally, it is my duty and pleasure to thank my husband, Nigel Orr, for his unfailing support, his infinite tolerance and for never being without a secret supply of chocolate.

Abbreviations

3Rs	replacement, refinement and reduction (of the use of animals in research)
4-ABP	4-aminobiphenyl
AAF	acetylaminofluorene
ADME	Absorption, Distribution, Metabolism and Excretion
AFB1	aflatoxin B1
AhR	arylhydrocarbon receptor
ALT	alanine aminotransferase
ASO	allele-specific oligonucleotide
ASPCR	allele-specific polymerase chain reaction
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the plasma concentration–time curve
BAC	bacterial artificial chromosome
BBB	blood-brain barrier
BMD	benchmark dose
bp	base pair
BrdU	bromodeoxyuridine
CAR	constitutive androstane receptor
cdk	cyclin-dependent kinase
cDNA	copy DNA
CEBS	Chemical Effects on Biological Systems
ChIP	chromatin immunoprecipitation
CHO	Chinese hamster ovary
CIN	cervical intraepithelial neoplasia
CITCO	6-(4-chlorophenyl)-imidazo[2,1-b]thiazole-5-carbaldehyde
CL_{INT}	intrinsic clearance
C_{MAX}	maximum (plasma) concentration
CNS	central nervous system
COMET	Consortium for Metabonomic Toxicology
CPMP	European Committee for Proprietary Medical Products
CYP	cytochrome P450

DDI	drug–drug interaction
DEHP	diethylhexylphthalate
DEN	diethylnitrosamine
DILI	drug-induced liver injury
DMBA	7,12-dimethylbenz(a)anthrancene
DMN	dimethylnitrosamine
DMSO	dimethyl sulphoxide
EC_{50}	concentration giving 50% of maximal effect
ECHA	European Chemical Agency
ECVAM	European Centre for the Validation of Alternative Methods
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ENU	ethylnitrosourea
EPA	US Environmental Protection Agency
ESC	embryonic stem cell
EST	embryonic stem cell test
EU	European Union
FABP	fatty acid binding protein
FDA	US Food and Drug Administration
floxed	flanked with loxP sites
GC	gas chromatography
GFP	green fluorescent protein
γGT	gamma glutamyl transpeptidase
GI	gastrointestinal
GLP	Good Laboratory Practice
GSH	glutathione
GST	glutathione *S*-transferase
GTP	guanosine triphosphate
GWAS	genome-wide association study
HCC	hepatocellular carcinoma
hERG	human ether-a-go-go related gene
HO-1	haem oxygenase 1
HPRT	hypoxanthine phosphoribosyltransferase
HRN™	Hepatic Reductase Null™
HTS	high throughput screening
i.p.	intraperitoneal
i.v.	intravenous
IC_{50}	concentration giving 50% inhibition
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ILSI	International Life Sciences Institute
iPSC	induced pluripotent stem cell
IVIVE	*in vitro–in vivo* extrapolation
kb	kilobase
kD	kilo Dalton
KEGG	Kyoto Encyclopedia of Genes and Genomes
K_M	Michaelis constant

K_{OW}	octanol-water partition coefficient
LC	liquid chromatography
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LD_{50}	dose giving 50% lethality
LDH	lactate dehydrogenase
LOAEL	lowest observed adverse effect level
LPS	lipopolysaccharide
MALDI	matrix-assisted laser desorption ionisation
MAPK	mitogen-activated protein kinase
MDCK	Madin-Darby canine kidney
MDR	multidrug resistance protein
MHLW	Japanese Ministry of Health, Labour and Welfare
MHRA	Medicines and Healthcare Products Regulatory Agency
MIAME	Minimum Information About a Microarray Experiment
MIAPE	Minimum Information About a Proteomics Experiment
MNU	methylnitrosourea
MOE	margin of exposure
mRNA	messenger RNA
MRP	multi-drug resistance-associated protein
MS	mass spectrometry
MTD	maximum tolerated dose
NADPH	nicotinamide adenine dinucleotide phosphate
NAT	*N*-acetyltransferase
NHS	UK National Health Service
NIEHS	US National Institute of Environmental Health Sciences
NIH	US National Institutes of Health
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
NTP	US National Toxicology Programme
OECD	Organisation for Economic Co-operation and Development
p.o.	perioral
PAH	polycyclic aromatic hydrocarbon
PAMPA	Passive Artificial Membrane Permeability Assay
P_{app}	apparent permeability
PB	phenobarbital
PBBK	physiologically based biokinetic
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PCN	pregnenlonone 16α-carbonitrile
PCR	polymerase chain reaction
PhIP	2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine
pK_a	acid dissociation constant
PPARα	peroxisome proliferater activated receptor α
PPD	*p*-phenylene diamine
PXR	pregnane X-receptor
QA	quality assurance
QC	quality control
QSAR	quantitative structure–activity relationship

QSPR	quantitative structure–permeability relationship
RFLP	restriction fragment length polymorphism
RHE	reconstructed human epidermis
RIVM	Netherlands National Institute for Public Health and the Environment
RSMN	reconstructed skin micronucleaus
RT-PCR	reverse transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
SELDI	surface-enhanced laser desorption/ionization
SHE	Syrian hamster embryo
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
SULT	sulphotransferase
SXR	steroid X receptor
$t_{1/2}$	half life
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCPOBOP	1,4-bis[2-(3,5-dichloropyridyloxy)]benzene
TGP	Toxicogenomics Project in Japan
T_M	melting temperature
TNF	tumour necrosis factor
TOF	time-of-flight
TPA	12-*O*-tetradecanoyl phorbol 13-acetate
TTC	threshold of toxicological concern
UDS	unscheduled DNA synthesis
UGT	UDP-glucuronyl transferase
ULN	upper limit of normal
UV	ultraviolet
V_{max}	maximum velocity