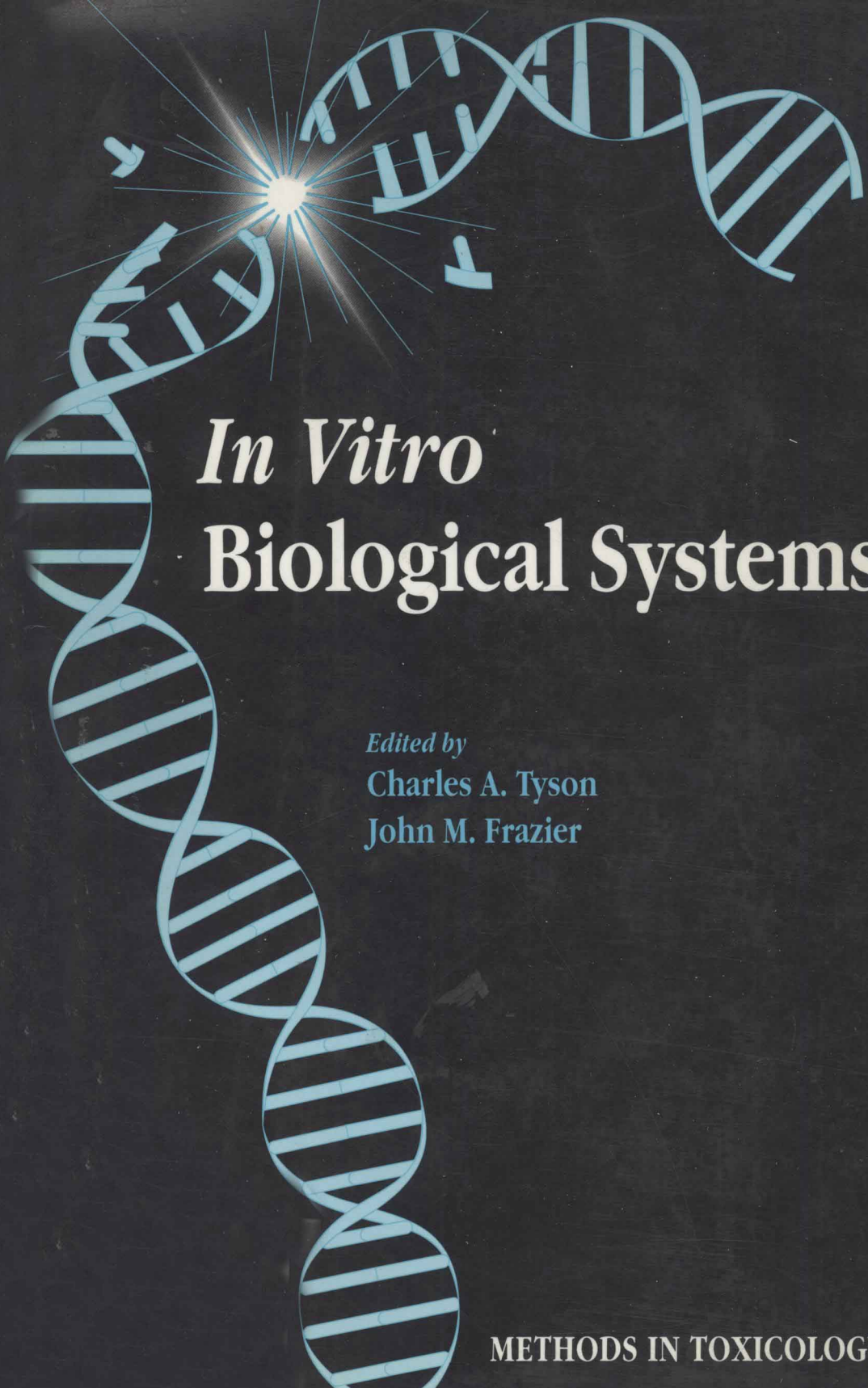

In Vitro Biological Systems

Edited by
Charles A. Tyson
John M. Frazier

METHODS IN TOXICOLOGY

Methods in Toxicology

VOLUME 1

Part A
In Vitro Biological Systems

Edited by

Charles A. Tyson
Biochemical Toxicology Program
SRI International
Menlo Park, California

John M. Frazier
School of Hygiene and Public Health
The Johns Hopkins University
Baltimore, Maryland

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Part A
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Series Editors

Charles A. Tyson
Biochemical Toxiology Program
SRI International
Menlo Park, California

Hanspeter Witschi
Institute of Toxicology and Environmental Health
University of California, Davis

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Mohamed B. Abou-Donia (70), Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710

Daniel Acosta (147, 244), Department of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712

Peter G. Aitken (1), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

Katherine L. Allen (262), Department of Toxicology, SRI International, Menlo Park, California 94025

Tak Yee Aw (193), Department of Physiology and Biophysics, Louisiana State University Medical Center, Shreveport, Louisiana 71130

Changli Bai (193), Department of Medicine, Emory University School of Medicine, Atlanta, Georgia 30322

Thomas K. Baumann (61), Departments of Surgery and Pharmacology, Oregon Health Sciences University, Portland, Oregon 97201

Anton M. Bennett (311), Division of Pathology and Toxicology, American Health Foundation, Valhalla, New York 10595

Benny L. Blaylock (455), Immunotoxicology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

J. L. Borowitz (82), Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

Klaus Brendel (222, 330), Department of Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona 85724

Robert E. Chapin (431), Developmental and Reproductive Toxicology Group, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Christine H. Chichester (123), Department of Anatomy, School of Veterinary Medicine, University of California, Davis, Davis, California 95616

Dennis W. Choi (46), Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110

Hyung Choi (27), Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637

Elinor M. Cockburn (385), Department of Health, London SW8 5NQ, England

Joel B. Cornacoff (467), Sterling Winthrop, Inc., Rensselaer, New York 12144

Lydia R. Cox (159), Division of Chronic and Biochemical Toxicology, E. I. du Pont, Haskell Laboratory, Newark, Delaware 19714

Jack E. Dabbs (348), Department of Toxicology, SRI International, Menlo Park, California 94025

Julio C. Davila (244), Department of Chemical Pharmacology, National Heart, Blood and Lung Institute, National Institutes of Health, Bethesda, Maryland 20892

Kathleen G. Dickman (339), Department of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794

Patricia A. Egner (525), Division of Toxicological Sciences, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205

Nelson Fausto (317), Department of Pathology and Laboratory Medicine, Brown University, Providence, Rhode Island 02912

Jacob N. Finkelstein (110), Department of Pediatrics, University of Rochester School of Medicine, Rochester, New York 14642

Karen J. Finney (202), Department of Pathology, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE1 4LP, England

Robyn L. Fisher (222), Department of Pharmacology, University of Arizona, Tucson, Arizona 85724

Scott L. Friedman (292), Department of Medicine, UCSF Liver Center Laboratory, San Francisco General Hospital, San Francisco, California 94110

Kevin Gaido (492), Department of Cellular and Molecular Toxicology, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina 27709

A. Jay Gandolfi (222), Department of Anesthesiology, University of Arizona, Tucson, Arizona 85724

Mark P. Goldberg (46), Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110

Peter Gouras (100), Department of Ophthalmology, Columbia University, New York, New York 10032

T. E. Gray (134), Laboratory of Pulmonary Pathobiology/National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Carol E. Green (262, 348), Department of Toxicology, SRI International, Menlo Park, California 94025

Christiane Guguen-Guillouzo (271), Unité de Recherches Hépatologiques, Institut National de la Santé et de la Recherche Médicale, 35033 Rennes, France

André Guillouzo (271), Unité de Recherches Hépatologiques, Institut National de la Santé et de la Recherche Médicale, 35033 Rennes, France

Gabrielle M. Hawksworth (385), Clinical Pharmacology Unit, Department of Medicine and Therapeutics, Division of Pharmacology, School of Biomedical Sciences, University of Aberdeen, Aberdeen AB9 2ZD, Scotland

Alfred Heller (27), Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637

Barbara Heller (27), Department of Mathematics, Illinois Institute of Technology, Chicago, Illinois 60616

R. Kelly Hester (169), Department of Medical Pharmacology and Toxicology, College of Medicine, Texas A&M University, College Station, Texas 77843

Hideyuki Hiraishi (182), Second Department of Internal Medicine, Dokkyo University School of Medicine, MIBU, Tochigi 321-02, Japan

J. Thomas Hjelle (330), Department of Basic Sciences, College of Medicine, University of Illinois, Peoria, Illinois 61656

Philip C. Hoffmann (27), Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637

Michael P. Holsapple (279, 482), Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Robert V. House (467), IIT Research Institute, Chicago, Illinois 60616

Gary E. Isom (82), Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

Kevin J. Ivey (182), Department of Gastroenterology, Veterans Affairs Medical Center, Long Beach, California 90822

Dean P. Jones (193), Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322

Marcia M. Jumblatt (94), Department of Ophthalmology and Visual Sciences, University of Louisville School of Medicine, Louisville, Kentucky 40292

Norbert E. Kaminski (279), Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Barbara W. Kemppainen (504), Departments of Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849

Thomas W. Kensler (525), Division of Toxicological Sciences, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205

Jane Knoth-Anderson (70), Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710

Carlos L. Krumdieck (222), Department of Nutrition Sciences, University of Alabama at Birmingham, University Station, Alabama 35294

Warren W. Ku (431), Developmental and Reproductive Toxicology Group, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Lawrence H. Lash (397), Department of Pharmacology, School of Medicine, Wayne State University, Detroit, Michigan 48201

Dennis A. Laska (411), Department of Acute, Ocular and Dermal Toxicology, Eli Lilly and Company, Greenfield, Indiana 46140

Michael I. Luster (455), National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Alvin M. Malkinson (123), Molecular Toxicology Program and Colorado Cancer Center, School of Pharmacy, University of Colorado, Boulder, Colorado 80309

Lazaro J. Mandel (339, 357), Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

Charlene A. McQueen (255), Department of Pharmacology and Toxicology, University of Arizona, Tucson, Arizona 85721

Elias Meezan (330), Department of Pharmacology, School of Medicine, University of Alabama at Birmingham, University Station, Alabama 35294

Francis B. Miley (123), Molecular Toxicology Program and Colorado Cancer Center, School of Pharmacy, University of Colorado, Boulder, Colorado 80309

Nancy A. Monteiro-Riviere (515), Cutaneous Pharmacology and Toxicology Center, North Carolina State University, Raleigh, North Carolina 27606

P. Nettesheim (134), Laboratory of Pulmonary Pathobiology/National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Charles G. Plopper (123), Department of Anatomy, School of Veterinary Medicine, University of California, Davis, Davis, California 95616

Kenneth S. Ramos (159, 169), Departments of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843

J. Edmond Riviere (515), Cutaneous Pharmacology and Toxicology Center, North Carolina State University, Raleigh, North Carolina 27606

Kamala Rose (46), Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110

Gary J. Rosenthal (455), Immunotoxicology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Charles E. Ruegg (357), In Vitro Technologies, Inc., Technology Enterprise Center, University of Maryland, Baltimore County, Baltimore, Maryland 21227

J. E. Rundhaug (134), Laboratory of Pulmonary Pathobiology/National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

T. W. Sadler (420), Department of Cell Biology and Anatomy, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Per O. Seglen (231), Department of Tissue Culture, Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway

Fredrick J. Seil (7), Neurology Research, Veterans Affairs Medical Center, Portland, Oregon 97201

John G. Simpson (385), Department of Pathology, University of Aberdeen, Aberdeen AB9 2ZD, Scotland

James L. Stevens (374), The W. Alton Jones Cell Science Center, Lake Placid, New York 12946

Mary L. Taub (366), Department of Biochemistry, State University of New York at Buffalo, Buffalo, New York 14214

Akira Terano (182), Second Department of Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

Nancy L. Thompson (317), Department of Medical Oncology, Rhode Island Hospital/Brown University, Providence, Rhode Island 02903

Charles A. Tyson (348), Biochemical Toxicology Program, SRI International, Menlo Park, California 94025

Lawrence W. Updyke (492), Medical Research Division, American Cyanamid Co., Pearl River, New York 10965

Allison A. Welder (147), Division of Medicinal Chemistry and Pharmacodynamics, College of Pharmacy, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

Paul H. Whiting (385), Department of Clinical Biochemistry, University of Aberdeen, Aberdeen AB9 2ZD, Scotland

Daniel Wierda (492), Lilly Research Laboratories, Eli Lilly and Company, Greenfield, Indiana 46140

Gary M. Williams (311), Division of Pathology and Toxicology, American Health Foundation, Valhalla, New York 10595

Patricia D. Williams (411), Department of Investigative Toxicology, American Cyanamid Co., Medical Research Division, Pearl River, New York 10965

Lisa Won (27), Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637

Steven C. Wood (482), Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Kyu Hwan Yang (279), Department of Life Sciences, Korea Advanced Institute of Sciences and Technology, Taejon 305-701, Korea

Guohong Zhang (374), The W. Alton Jones Cell Science Center, Lake Placid, New York 12946

S. Zhu (134), Laboratory of Pulmonary Pathobiology/National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Preface

New methods for evaluating the toxic effects of chemicals and drugs on biological systems are being developed at an ever-increasing pace. Recent advances in the biological, chemical, and physical sciences allow more detailed investigation of the mechanisms by which chemical agents cause organismic or cellular damage. There is a pressing need among researchers in the toxicology field for a series of authoritative texts that organize and present information on the latest experimental methodologies for reference.

Methods in Toxicology is a new series created to fill this need. These volumes are patterned after the highly regarded *Methods in Enzymology* series and are intended to provide comprehensive descriptions of state-of-the-art methods for investigating drug and chemical toxicity. We are pleased to be invited to compile the first volume in this series covering techniques for preparation and maintenance of *in vitro* cell and tissue systems for toxicological studies. Thematic material in this and future volumes will focus on mechanistic and risk assessment approaches to the evaluation of toxicity using *in vitro, in vivo,* and computational methods. We express our gratitude to Drs. M. W. Anders, Doyle Graham, and Emil Pfitzer for having laid the foundation for this series and provided the initial impetus.

In vitro methods is an appropriate subject for this inaugural volume. These methods have provided a valuable tool for toxicological research over the years. Historically, they have made significant contributions to our understanding of mechanisms of action of toxicants and pathways of xenobiotic metabolism. In this context, they have proved an indispensable resource for investigative toxicology. More recently, the value of *in vitro* systems as toxicity tests for chemical safety/hazard evaluation has been recognized and explored.

The objective of this volume is to provide both beginning and established researchers with basic techniques employed by widely recognized scientists to prepare and maintain the biological components of *in vitro* model systems. The compilation is not intended to be exhaustive but to provide a set of pivotal methods of value to research in the field of toxicology. Although *in vitro* studies in isolated organelles are important in toxicological research, these methods

have not been included in this volume. The editors assume that the reader is familiar with basic techniques in cell/tissue culture. If this is not the case, we suggest that the reader refer to one of the several useful books on this subject (1–3).

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John M. Frazier and Charles A. Tyson

Introduction

John M. Frazier and Charles A. Tyson

Although the term *in vitro* literally means in glass, *in vitro* toxicology generally refers to the study of toxicological phenomena in nonwhole animal models. This broader connotation for *in vitro* includes research studies utilizing isolated organs, tissue slices, explant cultures, and isolated primary cells as well as cell lines and subcellular fractions (e.g., microsomes, mitochondria, plasma membrane vesicles, etc.). These model systems have made major contributions to toxicological sciences, particularly in our understanding of mechanisms of toxicity, xenobiotic metabolism, and species differences in expressions of toxicity, to mention a few. In the last 10 years, the value of *in vitro* systems in toxicity testing has been widely recognized and explored. This recent focus on *in vitro* models, as a result of (i) rapid advances in biotechnology, (ii) economic costs (both in dollars and time) for adequate toxicological evaluation of new industrial chemicals and commercial products, and (iii) societal concerns for animal welfare, has led to the rapid development of new *in vitro* models for toxicological research. Whether the *in vitro* approach is employed as a model for investigative research or as a toxicity test for risk assessment, a critical component is the biological system. Successful preparation and maintenance of the appropriate cell, tissue, or organ for a particular scientific objective are essential for a satisfactory research outcome.

Before we describe the organization of this book further, it is important for the reader to understand the role of *in vitro* models in toxicological research. In general, the objective of any research activity is to develop new knowledge about a particular topic. This is usually accomplished by the application of the scientific methods of observation, hypothesis formulation, and hypothesis testing. The selection of the experimental system to conduct the hypothesis testing aspect of the study is determined mainly by the scientific question being asked (1). In many cases, observational studies *in vivo* generate specific hypotheses that can be tested in *in vitro* model systems. Once the decision has been made that an *in vitro* model may be the best scientific approach, the practical question becomes which *in vitro* model will best serve to test the proposed hypothesis.

The purpose of this book is to provide both new and established researchers

with a collection of some of the more important *in vitro* systems used in toxicological research today. It is not possible to catalogue in a single volume all existing *in vitro* models that are available for toxicological research. Therefore we have restricted this book to methods that focus on slices, explant, primary cell, and cell line cultures. In addition, because of the rapid evolution of new model systems we have attempted to include models that have a broader acceptance or potential for acceptance within the toxicological research community. The models have been divided into categories on the basis of the major organ systems which they represent since this seems most appropriate for the traditional organization of toxicological research. However, the classification of models by associated organ should not imply any conceptual restriction in terms of the applications of various model systems in toxicological research. For example, toxicological studies in Kupffer cells (a liver-associated cell type) may be applicable to certain aspects of the immune system because of their macrophage origin.

The selection of an appropriate model to investigate a particular scientific question or test a particular hypothesis is not a trivial exercise. Several important concepts must be taken into consideration—characterization, standardization, and validation of the experimental model. Characterization refers to the measurement of a set of observable parameters (morphological, physiological, or biochemical) that can be used to accurately define the biological model in terms of purity and integrity and its functional state. The system can be standardized by setting specific criteria which these parameters must meet in order to be acceptable for a particular research activity. Good research must utilize well-characterized biological models that meet standardized criteria. In the context of investigative research, validation of a method becomes a reality only when it is demonstrated that the understanding provided by the model is applicable to the *in vivo* situation under investigation. Thus, a particular *in vitro* model may be extremely useful for one research problem (and thus be considered validated) and have little or no applicability to other research problems. When *in vitro* models are used for toxicity testing, the model is usually validated for a restricted set of well-documented, reference chemicals and then applied to a broad range of test materials. The validation of the model is not confirmed in each and every case but is assumed on the basis of the performance of the model for the reference chemicals. This difference between validation for investigational research and validation for toxicity testing has caused considerable confusion in the scientific community (2).

There are no hard and fast rules for selection of an *in vitro* model for a particular investigation; however, the researcher must be aware of various information—what models are available, how they are characterized and standardized,

for what kind of scientific questions have they been validated—in order to make a more rational decision. It is hoped that this book will aid the toxicological researcher in this difficult task.

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