

Non-Animal Techniques

in

Biomedical and Behavioral Research and Testing

**Michael B. Kapis
Shayne C. Gad**

R-33
K17

9462531

Non-Animal Techniques

in

Biomedical and Behavioral Research and Testing

**Michael B. Kapis
Shayne C. Gad**



E9462581



LEWIS PUBLISHERS

Boca Raton Ann Arbor London Tokyo

Library of Congress Cataloging-in-Publication Data

Non-animal techniques in biomedical and behavioral research and testing / edited by Michael Kapis and Shayne C. Gad.

p. cm.

Includes bibliographical references and index.

ISBN 0-87371-504-7

1. Biology, Experimental. 2. Animal experimentation. 3. Human experimentation in medicine. 4. Toxicity testing—In vitro.

5. Medicine, Experimental—Data processing. 6. Medicine, Experimental. I. Kapis, Michael. II. Gad, Shayne C., 1948—

R852.N65 1993

619—dc2

92-45746

CIP

COPYRIGHT © 1993 by LEWIS PUBLISHERS

ALL RIGHTS RESERVED

This book represents information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Every reasonable effort has been made to give reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

All rights reserved. Authorization to photocopy items for internal or personal use, or the personal or internal use of specific clients, is granted by CRC Press, Inc., provided that \$.50 per page photocopied is paid directly to Copyright Clearance Center, 27 Congress Street, Salem, MA 01970, USA. The fee code for users of the Transactional Reporting Service is ISBN 0-87371-504-7/93/\$0.00+\$.50. The fee is subject to change without notice. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

The copyright owner's consent does not extend to copying for general distribution, for promotion, for creating new works, or for resale. Specific permission must be obtained from CRC Press for such copying.

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

PRINTED IN THE UNITED STATES OF AMERICA

1 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

Non-Animal Techniques
in
Biomedical and Behavioral
Research and Testing

Preface

Within the last two decades, there has been a steadily increasing number of researchers, students, and educators investigating and utilizing alternative-to-animal methods in biomedical and psychological research, testing, and education. Some primary incentives for this interest in alternatives are (1) many recent advances in biotechnology, (2) ethical issues involving animal experimentation, (3) accuracy and precision, (4) expedience, and (5) cost effectiveness.

In 1959, Russell and Birch defined alternatives as “Refinement, Reduction, and Replacement”, commonly known as the 3 Rs. This volume will focus primarily on two of the 3 Rs — reduction and replacement. Therefore, we define an alternative as any method (primarily non-animal) that either reduces or replaces the need for animal models in biomedical and behavioral research, testing, and education. The exceptions are observational, painless, stress-free, and noninvasive laboratory investigations, as well as the study of animals in their natural environments (ethology).

Alternatives are now involved in every aspect of biomedical and behavioral research, testing, and education. Many of the recent advances in AIDS, heart disease, cancer, stroke, cystic fibrosis, Alzheimer’s disease, drug designs, multiple sclerosis, Parkinson’s disease, schizophrenia, etc., have benefitted and have been accomplished by the use of alternative methodologies.

In spite of this success, however, many scientists either are unaware or maintain “outdated” views of alternatives, adhering to the traditional practice of using animal models. The purpose of this volume is to inform the reader of the numerous potential applications that alternatives offer to biomedical and behavioral investigations. In order to accomplish this, we invited recognized leading scientists from industry and academia to write on their areas of expertise pertaining to alternatives. Emphasis was placed both on the application and on the strengths and weaknesses of the methods. An exhaustive but unsuccessful effort was made to enlist experts in the areas of physicochemical techniques, medical microbiology, and human autopsies. Since these are important areas of research and testing, we wrote brief chapters on these subjects and included extensive references.

Alternatives have given new meaning to “never say never”. What a decade ago was thought to be “impossible” is now often “probable”. Only a lack of imagination or ingenuity stands as an impediment to an ever-increasing utilization of alternative methods in biomedical and behavioral investigations.

Michael B. Kapis
Shayne C. Gad

Acknowledgments

I wish to thank everyone involved with supplying information and support for *Non-Animal Techniques in Biomedical and Behavioral Research and Testing*. I wish to extend special appreciation to Rowland Mitchell for proofreading and suggestions, to Kevin Winterfield for inspiration, to the librarians of the San Francisco Bay Area for their assistance, to Jon Lewis and staff at Lewis Publishers for their support and their unbelievable patience, and to Irene, Eleanor, and Cynthia for word processing.

Finally, I owe a special thanks to my wife, Luz, and my son, Christopher, for putting up with me during the lengthy creation of this book.

Michael B. Kapis

To Tine, and a special time. May it somehow and somewhere go on forever. And may the Lord always keep her in the sunshine.

Shayne C. Gad

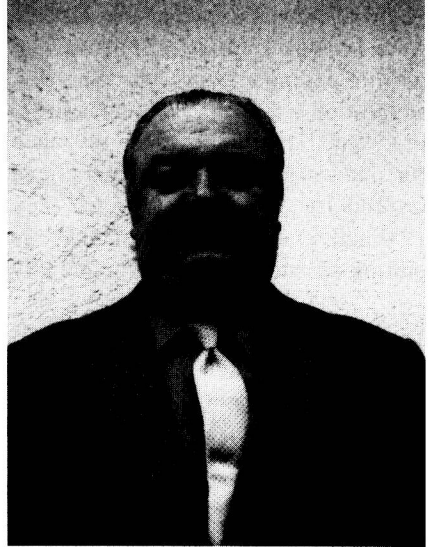
The Editors

Michael B. Kapis, B.A., B.S., earned his B.A. in Physics from San Jose State University, where he also earned his B.S. and completed his coursework for the M.S. in biological science. He has a wide range of interests in the animal sciences.

During the time he was pursuing his degree in astrophysics, Mike became very interested in techniques and devices that might prevent animal-vehicle collisions. In particular, he is interested in ultrasonic sound and fluorescent markings as possible collision-deterrent mechanisms. His master's thesis involves the effects of alarm signals on deer movements. This interest in animal-vehicle accidents later led him to change his major from astronomy to biology.

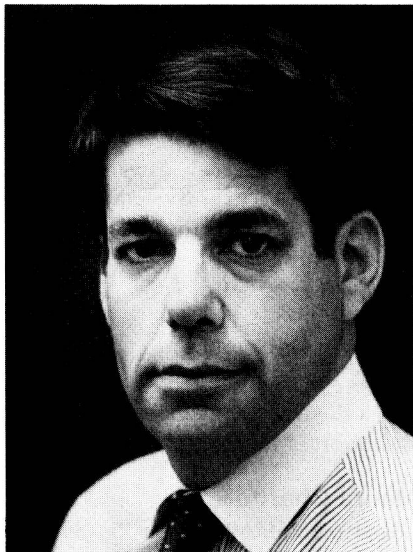
In 1983, while visiting Davao City in the Philippines, Mike became involved with the plight of the endangered Philippine eagle. He later formed a nonprofit organization—Save The Philippine Eagle Fund—and since that time, he has focused his attention on conserving and restoring the rainforest habitat of the Philippine eagle.

Throughout his studies in biology, Mike has had an interest in the use of animals in research, testing, and education. He was concerned about the amount of needless duplication in animal experimentation. Later, he became aware that there were alternative-to-animal methods available. He selected the topic of alternatives for a graduate seminar presentation. He assembled many articles on alternatives while preparing for the seminar, which was well received. In 1986, Mike and a fellow graduate student, Kevin Winterfield, began publishing the quarterly *Alternatives To Animals Newsletter*, geared for the research and academic community.



Shayne C. Gad, Ph.D.,

is Director of Medical Affairs Product Support Services at Becton Dickinson, Research Triangle Park, North Carolina. His interests include neurotoxicology, alternative methods and models, dermal and immune toxicology, and statistics in toxicology. He has published more than 230 abstracts, articles, papers, chapters, and books in the field of toxicology. He is on the editorial boards of the *Journal of Applied Toxicology*, the *Journal of Fire Science*, and the *Journal of Acute Toxicology*; he is also Editor-in-Chief for *Toxicology Methods*. He has served on the National Institute of Standards and Technology Combustion Toxicology



Task Force, the Consumer Product Safety Commission Toxicology Advisory Board, and on the SOT Animals in Research Nominations and Placement Committees; he has also served with trade association groups for Nylon 6, chrome chemicals, cyclohexanone, ketones, and phthalates. He is past president of the American College of Toxicology and is editor of the SOT Reproductive and Developmental Toxicology Newsletter.

Dr. Gad has lectured at Texas, Kansas, Rutgers, Johns Hopkins, and Pittsburgh, has served on several Ph.D. thesis committees, and is a grant reviewer for the Center for Alternatives to Animal Testing at Johns Hopkins University; he also established and taught a bachelor program in toxicology at the College of St. Elizabeth.

Dr. Gad serves on the National Institutes of Health (NIH) Occupational Safety and Health Study Section. He is a member of the Society of Toxicology, the American College of Toxicology, the Teratology Society, the Biometrics Society, and the American Statistical Association, and he is a Diplomate of the American Board of Toxicology.

Contributors

C. L. Alden

Product Safety and Metabolism
Searle
Skokie, Illinois

Sarah Barron

University of Texas System
Center for High Performance
Computing
Austin, Texas

Charles Bauer, Jr.

NovaScreen®
Nova Pharmaceutical Corporation
Baltimore, Maryland

Maura Charlton

Department of Molecular Psychiatry
Yale University School of Medicine
New Haven, Connecticut

Jesse Driver

University of Texas System
Center for High Performance
Computing
Austin, Texas

Shayne C. Gad

Medical Affairs Product Support
Services
Becton Dickinson
Research Triangle Park, North
Carolina

Alan M. Goldberg

Center for Alternatives to Animal
Testing
School of Public Health
Johns Hopkins University
Baltimore, Maryland

Virginia C. Gordon

In-Vitro International
Irvine, California

Andrew J. Greenshaw

Department of Psychiatry
University of Alberta
Edmonton, Alberta, Canada

Robert Harkness

University of Texas System
Center for High Performance
Computing
Austin, Texas

Michael B. Kapis

Alternatives To Animals
San Jose, California

Jennifer L. Kelsey

Division of Epidemiology
Stanford University School of
Medicine
Stanford, California

Peter H. Klopfer

Department of Zoology
Duke University
Durham, North Carolina

John M. Last

Epidemiology and Community
Medicine
University of Ottawa
Ottawa, Ontario, Canada

Peter G. Morris

Magnetic Research Center
Department of Physics
University of Nottingham
Nottingham, England

Susan Parker

School of Public Health
Columbia University
New York, New York

Paul S. Prueitt

Neural Network Research Facility
Department of Physics
Georgetown University
Washington, D.C.

Paul M. Sweetnam

Exploratory Medicinal Chemistry
Pfizer Central Research
Groton, Connecticut

Fang Wang

University of Texas System
Center for High Performance
Computing
Austin, Texas

Frederick Wehr

Consultant
500 Edgevale Road
Baltimore, Maryland

Matthew Witten

University of Texas System
Center for High Performance
Computing
Austin, Texas

John Yam

Cosmetics & Fragrance Worldwide
The Procter & Gamble Company
Cincinnati, Ohio



Table of Contents

Chapter 1 Alternatives to Whole Animal Testing

Alan Goldberg and Frederick Wehr

1.0 Introduction	1
1.1 Why Test?	2
1.2 A New Compound	2
1.3 Looks Promising	3
1.4 Clinical Testing	3
1.5 Gaining Regulatory Approval	4
1.6 Animal Testing	4
1.7 Alternatives—Refinement, Reduction, Replacement	5
1.8 Acute Toxicity Testing	7
1.9 The Promise and Potential of <i>In Vitro</i> Methods	8
References	8

Chapter 2 Applications of *In Vitro* Methods for the Cosmetic, Household Products, and Pharmaceutical Industries

Virginia C. Gordon

2.0 Introduction	11
2.1 Current <i>In Vitro</i> Methods	12
2.2 Description of Current <i>In Vitro</i> Methods	13
2.3 Applications of <i>In Vitro</i> Methods	17
2.4 Summary	21
References	22

Chapter 3 *In Vitro* Testing Program at Procter & Gamble

John Yam and C. L. Alden

3.0 Introduction	27
3.1 Acute Oral Toxicity	28
3.2 Eye Irritation	29
3.3 Skin Irritation	32
3.4 Contact Hypersensitivity and Photoallergy	32
3.5 Respiratory Toxicology	33
3.6 Genotoxicity and Carcinogenicity Testing	34
3.7 Developmental Toxicology	35
3.8 Noninvasive Techniques	35
3.9 Computer Applications	36
3.10 Other Applications	37
3.11 Validation of Alternative Methods	38
3.12 Conclusions	39
References	40

Chapter 4 *In Vitro* Techniques for Use in Drug Discovery

Paul Sweetnam, Charles Bauer, Jr., and Maura Charlton

4.0	Introduction	43
4.1	Radioligand Binding	43
4.2	Cell Culture	47
4.3	Radioligand Binding and Drug Discovery	51
4.4	Conclusions	59
	References	59

Chapter 5 Structure–Activity Approaches as an Alternative to Animal Testing for Predicting Toxicity in Man

Shayne C. Gad

5.0	Introduction	63
5.1	Basic Assumptions	65
5.2	Molecular Parameters of Interest	65
5.3	SAR Modeling Methods	66
5.4	Applications in Toxicology	69
	References	70

Chapter 6 Biological Studies in Psychiatry: Neurochemical Measurements with Human Subjects

Andrew J. Greenshaw

6.0	Introduction	73
6.1	The Advent of Chemotherapy in Psychiatry	74
6.2	Approaches to Neurochemical Measurements in Human Subjects	74
6.3	The Measurement of Neuroactive Compounds in Human Body Fluids	76
6.4	Measurements of Receptor-Related Mechanisms— A Possible Key to Understanding Actions of Psychotherapeutic Drugs	77
6.5	Studies Related to Antidepressant Drug Action	78
6.6	Antipsychotic Drug Action— The Advent of PET Analysis	79
6.7	Conclusion	81
	References	81
	Additional Reading	85

Chapter 7 Network Models in Behavioral and Computational Neuroscience

Paul S. Prueitt

7.0	A Brief History of Neural Networks— The Early Epoch	87
7.1	Neural Networks—The Middle Epoch	91
7.2	Discrete and Continuous ANNs	93

7.3 The Impending Paradigm Shift.....	94
7.4 Does Neural Network Research Require a New Notion of Scientific Inquiry?	97
7.5 Summary	99
References	99

Chapter 8 Ethology and Noninvasive Techniques

Peter H. Klopfer

8.0 History of Animal Behavior Studies	103
8.1 Goals of Behavior Studies	105
8.2 Ethological Techniques	106
8.3 Summary	112
References	112
Additional Reading	112

Chapter 9 Computational Modeling of Biological/Medical Systems

Matthew Witten

9.0 Introduction	115
9.1 Modeling/Simulation of Living Systems	116
9.2 What is High-Performance Computing?	118
9.3 Visualization	120
9.4 Computational Genetics	122
9.5 Computational Cell Biology	123
9.6 Computational Physiology	124
9.7 Reproductive Biology	124
9.8 The Heart of Supercomputing—Cardiac Dynamics	125
9.9 The Nervous System	126
9.10 Computing the Kidney	127
9.11 Modeling the Dynamics of the Body	127
9.12 Patient-Based Physiological Simulation	128
9.13 Project Human	128
9.14 Computational Population Biology	129
9.15 Computational Chemistry	130
9.16 Agricultural/Veterinary Applications	131
9.17 Computational Dentistry	131
9.18 Matrix of Biological Knowledge	134
9.19 Conclusions	135
References	136

Chapter 10 The Human Genome Project: An Overview of Computational Issues in Molecular Biology and Genetics

*Sarah Barron, Matthew Witten, Robert Harkness,
Fang Wang, and Jesse Driver*

10.0 The Promise of the Human Genome Project	145
--	-----

10.1	The Human Genome Effort— Not Just Computers, Not Only Animals	146
10.2	The Data	146
10.3	Biology	148
10.4	Sequence and Search Algorithms	150
10.5	Visualization	152
10.6	Communication	153
10.7	Hardware	153
10.8	Summary	155
	References	155
	Additional Reading	158

Chapter 11 Epidemiology as an Alternative to Animal Research

Jennifer L. Kelsey and Susan Parker

11.0	Introduction	165
11.1	Descriptive Epidemiology	166
11.2	Analytic Epidemiology	166
11.3	Epidemiologic Methods in Studying Behavioral Variables	173
11.4	Other Useful Epidemiologic Concepts	173
11.5	Criteria for Causation	175
11.6	AIDS: An Epidemiologic Paradigm	178
11.7	Sample Size Considerations	179
11.8	Future Directions in Epidemiology	181
	References	182

Chapter 12 Alternatives to Animals in Preventive Medicine

John Last

12.0	Introduction	187
12.1	Moral Dilemmas of Public Health and Preventive Medicine	188
12.2	Disease Control Programs and Animal Diseases	189
12.3	Interconnections Between Animal and Human Health	189
12.4	What is an Animal?	190
12.5	Alternatives to Animals in the Practice of Preventive Medicine	192
	References	193

Chapter 13 Human Autopsies in Biomedical Research

Michael B. Kapis

13.0	Introduction	195
13.1	The Role of Autopsies in Modern Biomedical Research	196

13.2	Results of 85 Years of Autopsies in Trieste, Italy	198
13.3	The Present-Day Decline of Autopsies	199
13.4	Future of Autopsy Research	199
	References	199

Chapter 14 Medical Microbiology

Michael B. Kapis

14.0	Introduction	207
14.1	A Brief History of Discoveries in Medical Microbiology	208
14.2	Microbial Control	208
14.3	Introduction to Medical Bacteriology	209
14.4	A List of Some of the Important Medical Bacteria	209
14.5	Introduction to Medical Mycology	210
14.6	A List of Some of the Medically Important Mycoses	210
14.7	Introduction to Medical Virology	211
14.8	A List of Some of the Medically Important Viruses	211
14.9	Diagnostic Methods in Medical Microbiology	212
	References	213

Chapter 15 Physicochemical Techniques in Biological Research and Testing

Michael B. Kapis

15.0	Introduction	217
15.1	Chromatography	217
15.2	Spectroscopy	219
	References	221

Chapter 16 Magnetic Resonance and the Use of Animals

Peter G. Morris

16.0	Imaging Methods	237
16.1	Magnetic Resonance Imaging— Basic Principles	238
16.2	Magnetic Resonance Imaging— Clinical Applications	243
16.3	Magnetic Resonance Imaging— Animal Studies	245
16.4	Magnetic Resonance Spectroscopy	247
16.5	Future Developments	250
	References	252

Index	255
------------------------	-----

CHAPTER 1

Alternatives to Whole Animal Testing

Alan Goldberg and Frederick Wehr

1.0 Introduction

The Center for Alternatives to Animal Testing (CAAT), at the Johns Hopkins University School of Hygiene and Public Health in Baltimore, MD, was founded in 1981. It began as a cooperative venture of the School and the Cosmetic, Toiletry, and Fragrance Association, a body determined to develop ways of testing the safety of their products without having to rely so extensively on expensive, time consuming, and socially sensitive testing on live animals. The School's participation is a natural extension of its ongoing efforts in toxicology—the study of any substance, drug, or product that might be harmful, however mildly—and its intensive and world-wide pursuit of solutions to public health problems.

Today, CAAT is funded by almost 70 companies in the food, drug, cosmetics, consumer products, computer and petrochemical industries, and by individuals that support its mission.

In the years since the founding of the Center, substantial progress has been made toward reducing the number of animals necessary in product testing.¹ It must be recognized at the outset, however, that such institutions as the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) are charged with guarding the public from intrusion into the marketplace of potentially

harmful chemicals, and *in vivo* (live animal) testing is the proven and accepted method from which these agencies cannot depart without the most positive proof that any *in vitro* alternative testing method proposed is just as good if not superior to *in vivo* testing.²

1.1 Why Test?

All testing of any substance to which humans are to be exposed—whether it be a drug, a food preservative or additive, a cosmetic product, one for household use, or whatever—is conducted with two goals. Is the product effective? Can the product be used safely? The investment by American industry in answering these questions is in the billions of dollars annually. Nowhere is the scope and intensity of this research greater than in the drug industry. The reader, we believe, will gain a better understanding of the place and the importance of animal testing if we first review the procedures followed in the development of a new drug and the questions and quandaries posed to researchers as they go about their work.³

Scientists in drug research invariably begin with a hypothesis. They assume that a new molecule or a modification of a molecule known to have certain effects on the human body might lead to a medication which would be an improvement over some existing drug or, even more dramatic, provide a cure for a disease to date unaffected by medications. The laws of chemistry limit the extent to which molecular modification is possible. Within those limits, the researchers and their colleagues devise methods to create the molecule envisioned bearing in mind, all along, that the number of potentially helpful molecules—the laws of chemistry notwithstanding—is enormous and that the track they have elected to follow commits the firm to a huge investment. Whether it will be commercially successful is unknown. It is estimated that only 1 in about 10,000 compounds formulated turns out to be helpful and that the average cost of developing one new drug may exceed \$150 million.

1.2 A New Compound

Once the molecule has been synthesized, several questions arise. Is it more effective than an existing drug? What will be its side effects and are they greater or less than those of an existing drug? Might it prevent the onset of disease? And, more dramatically, might it treat a disease which is now untreatable or cure a disease which is now incurable? Let us suppose that the new molecule has been formulated, that it is stable, and that the rationale for its creation stands up to intellectual examination. Has it already been patented by another firm? If so, did that competitor devise the molecule with the same objective in mind? Maybe their researchers were seeking a cure for a different disease. If so, what are the implications?

After the new compound has been created, it is examined by pharmacologists who must determine whether it has any value. At this point, the testing of the drug