Non-Animal Techniques in Biomedical and Behavioral Research and Testing

Michael B. Kapis
Shayne C. Gad

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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In spite of this success, however, many scientists either are unaware or maintain "outdated" views of alternatives, adhering to the traditional practice of using animal models.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

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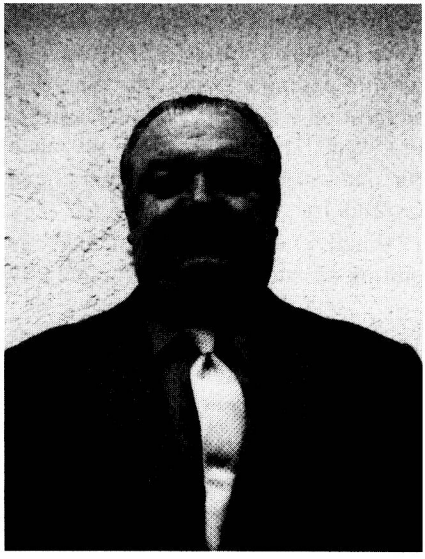
Finally, I owe a special thanks to my wife, Luz, and my son, Christopher, for putting up with me during the lengthy creation of this book.

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

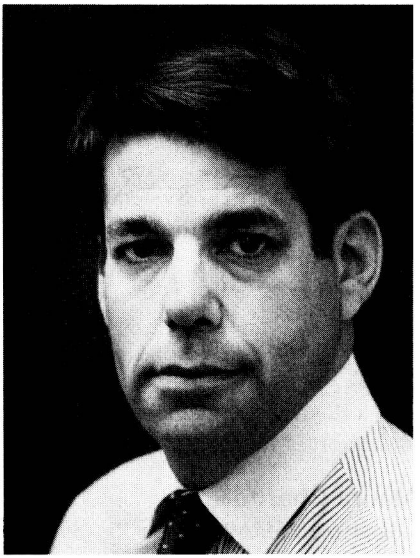


Michael B. Kapis, B.A., B.S., earned his B.A. in Physics from San Jose State University, where he also earned his B.S. and completed his coursework for the M.S. in biological science. He has a wide range of interests in the animal sciences.

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.

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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

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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