

## Preface

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The topic of apoptotic cell death has received a lion's share of attention, especially within the last 15 years. This heightened interest likely results from recent recognition of the relevance of apoptosis to a variety of scientific disciplines; pharmacology and toxicology are no exceptions. The major goals for toxicologists who study apoptosis, however, often differ from the investigative goals of other disciplines. In many cases, a toxicologist or pharmacologist is faced with the task of quantifying an apoptotic response or assessing, in a quantitative way, the mechanism by which a chemical or drug interacts with apoptotic signaling factors. Frequently, the final outcome of this task is to apply results to safety evaluation or assess relevance to environmental exposures. Although there are several publications that review and instruct the reader about detection of apoptosis, many of these texts do not pair the methods used for evaluating apoptosis with the need to evaluate safety or risk assessment. The primary aim of this book is to review methods that can be used by toxicologists, pathologists, or pharmacologists in the analysis of chemical-induced apoptosis. *Apoptosis Methods in Pharmacology and Toxicology: Approaches to Measurement and Quantification* provides a concise source of information on the detection, mechanisms, and quantification of apoptosis that is useful for the design of toxicology and pharmacology studies.

The range of methods covered may seem surprisingly narrow at first glance, but there are few methods that have proven to have broad application to numerous tissue and cell types or that can be applied to unknown induction mechanisms. The number of methods for detection may also seem small compared with the exciting and abundant activity in apoptosis research over the past 10 years. For example, PubMed lists 19,167 publications with apoptosis in the title between 1991 and 2001. Since research in the area of apoptosis has yielded reports of an overwhelming number of inducing agents, regulatory factors, and mechanisms, one might ask why the number of biological assays for measurement of apoptosis is comparatively low. One reason may be that despite the plethora of molecular factors and events that have been found to participate in the apoptotic process, only a few of these appears to have the sensitivity, specificity, and universal application

required to merit acceptance as reliable biological assays for measurement of apoptosis. The search for molecular and biochemical events in apoptosis common to most cell types and induction pathways has resulted in assays that are largely based on the biochemical mechanisms that regulate the morphologic features of apoptosis. Even after years of use, several of the current methods used to identify or quantify apoptosis are still developing because of limited testing in different cell types, in experimental models, or in tissue sections.

In *Apoptosis Methods in Pharmacology and Toxicology*, meaningful and cutting edge chapters were contributed by authors with substantial knowledge of the technical challenges and caveats of methods used for analysis of apoptosis. Each chapter emphasizes how the method can be used in evaluation of apoptosis, the limitations of the method, and how the technique may be applied for large-scale screening applications. In the introductory chapter, there is a brief overview of study design and approaches to mechanistic studies of toxicant-induced apoptosis. The remaining chapters provide a concise source of information on detection and quantification of apoptosis that can be incorporated into the design of toxicological evaluations. In Chapter 2, Martin Poot, Robert H. Pierce, and Terrance J. Kavanagh review the flow cytometric and fluorometric methods of quantifying and characterizing apoptosis. Measurement of several biochemical features of apoptosis are discussed and protocols are provided for the measurement of cell-cycle stage-specific apoptosis and the simultaneous measurement of mitochondrial membrane potential, and reduced thiol and NAD(P)H levels. Chapter 3, contributed by Zbigniew Darzynkiewicz, Elzbieta Bedner, and Piotr Smolewski, is *the only comprehensive review* to date on the application of laser scanning cytometry in analysis of apoptosis. This relatively new and powerful method is discussed and detailed protocols are provided.

Chapter 4, contributed by Matthew A. Wallig, Curtis M. Chan, and Nancy A. Gillett, emphasizes challenges to tissue-based methods and reemphasizes the need to keep morphologic assessment of apoptosis as a "gold standard." Immunocytochemical approaches to the measurement of several biochemical and molecular endpoints in tissue sections are discussed. Quantification and qualitative analysis of morphology is also emphasized, along with quantification. This section will be highly useful for those carrying out studies in whole animal models, in contrast to cell culture systems.

DNA microarray technology is reviewed in Chapters 5 and 6. Chapter 5 by Helmut Zarbl reviews microarray analysis as a general technique, and Chapter 6 by Richard W. E. Clarkson, Catherine A. Boucher, and Christine

J. Watson focuses on the application of microarray technology in the measurement of apoptosis. Finally, relatively new ELISA techniques are described in detail in Chapter 7 by Calvin F. Roff and colleagues. This chapter is unique in that it describes approaches that can be applied as high throughput screens for functional quantification of protein or chemical inhibitors that target active caspases and the Bcl-2 family of proteins.

*Apoptosis Methods in Pharmacology and Toxicology* is expected to serve as a useful reference for all scientists who face the challenge of identifying apoptosis, or elucidating mechanisms of drug-induced injury, as well as those scientists bewildered by the abundant flow of new information on apoptosis whose practical application is difficult to discern. This volume should equally serve as a reference for any laboratory that has a general interest in studying apoptosis. In such an evolving field, new developments are continually being reported. It is recommended that everyone with a serious interest in apoptosis and cell death make use of one of the web-based discussion groups or attend conferences or workshops on the topic to stay informed about research that may have application to research questions in their labs.

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