INTRODUCTION

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Toxicology is defined variously as: "a science that deals with poisons and their effect" and "the scientific study of the characteristics and effects of poisons" [1, 2]. Rather dramatically, the emphasis is on "poisons"; a more inclusive definition of toxicology is, in our view, "the study of symptoms, mechanisms, treatments, and detection of poisoning, especially the poisoning of people." Within this context, toxicology has a long, checkered history, which is described in an interactive online poster, which has been produced by Gilbert and Hayes [3]. This poster describes the principal milestones in the evolution of toxicology and effectively illustrates the point that, for many years, toxicology was indeed principally concerned with the use of and protection from, exposure to poisons. It was not until the sixteenth century that Paracelsus highlighted the link between poisons and "remedies" [3]. With the passage of time, this "preindustrial" view of toxicology gave way to the modern "postindustrial" era of toxicology. As a result, the toxicological sciences have matured and expanded to include a range of specific subdisciplines of toxicology as follows:

- Clinical toxicology, the diagnosis and treatment of poisonings,
- Forensic toxicology, the use of analytical chemistry, pharmacology, and clinical chemistry to aid medicolegal investigation of death, poisoning, and drug use,
- Industrial or occupational toxicology, which deals with potential harmful effects of materials, products, and wastes on health and working environments,
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- **Environmental toxicology**, the study of the potential effects upon organisms of the release of materials derived from human activities into the natural environment and
- **Pharmaceutical toxicology**, the study of the potential effects on organisms of novel or established pharmaceuticals.

This book focuses on pharmaceutical toxicology and, in particular, nonclinical toxicology. Traditionally, nonclinical toxicology has had a bad image within pharmaceutical companies. This is often due to a poor understanding of the role of nonclinical toxicology in drug development. The regulatory guidelines that govern the design and conduct of toxicity studies still require, in most cases, that adverse events are produced in studies, or at a minimum, that very high doses (relative to clinical doses) be tested. As a result, toxicologists were/are seen as “drug killers,” or colleagues who conduct animal studies at unrealistically high doses of the test compound. In recent years, reforms within pharmaceutical companies, driven by changing scientific, regulatory, and economic environments, have meant that there is a greater interaction between different areas of a drug development organization. Consequently, there is increased understanding of the role of toxicity studies within drug development. Not only is toxicology, and the toxicological scientist, an integral part of the identification of drug candidates, structural optimization, and lead candidate selection, but it is a cornerstone of managing attrition. Yes, toxicology can “kill” a compound, but ideally, they will be compounds with unacceptable and/or unmanageable toxicities, and this attrition will occur as early in the development cycle as possible. This is good for the patient and is good economics. Nevertheless, on occasion, despite the best efforts of all those involved, a drug has to be withdrawn from use. Consider the case of Vioxx (rofecoxib), a COX-2 selective nonsteroidal anti-inflammatory drug (NSAID). This class of drugs was developed as a safer alternative to mixed COX-1/COX-2 NSAIDs such as aspirin, ibuprofen, and naproxen. It is now believed that all NSAIDs, when taken chronically, produce an increased risk of gastrointestinal bleeding and liver and kidney toxicity. In addition to problems typically associated with NSAIDs, several studies questioned the cardiovascular safety of Vioxx. In 2000, the Vioxx Gastrointestinal Outcomes Research (VIGOR) study, which compared Vioxx and naproxen, found that the risk of cardiovascular problems, including heart attack, chest pain, stroke, blood clots, and sudden death, was more than two times higher in the Vioxx group than in the control group and five times the risk of heart attack when compared to patients taking naproxen. Subsequently, the U.S. Food and Drug Administration (FDA), based on the analysis of the medical records of 1.4 million patients, suggested that Vioxx may have contributed to an additional 27,785 heart attacks or sudden cardiac deaths from 1999 to 2003. Because of these findings and data from additional studies, Vioxx was (voluntarily) withdrawn from the market by the manufacturer in 2004 [4]. It is worth noting that this withdrawal occurred despite the fact that many patients derived great benefit from this drug. Hopefully, in the future emerging technologies will help to target the use of drugs such as Vioxx to individual patients who have a maximal benefit/risk profile and in this way avoid the loss of valuable drugs to patients.
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Traditionally, nonclinical–toxicological assessment has been based largely on data derived from animal studies; this has all the well-known advantages and inconveniences associated with the use of animals. There is increasing pressure to reduce, if not eliminate, the use of animals for scientific experiments and to reduce the cost and time taken to develop new drugs. Ideally, therefore, animal toxicity studies should be replaced by a series of robust, highly predictive, low-cost, and simple to conduct in vitro and in silico (computational) studies. Much progress has been made in recent years toward this goal; however, we are still a long way from achieving this ideal. A range of in vitro studies, some of which are accepted by regulatory authorities, are now available to toxicologists; for example, the use of the 3T3 cell assay to test for phototoxicity potential [5]. In recent years, there have been great advances in decoding genes and DNA sequences from a number of organisms, a task that has been facilitated by the development of techniques such as microarrays [6, 7] and array-based comparative genomic hybridization [8, 9]. At present, one million sites in any individual’s genomic DNA can be simultaneously interrogated, which facilitates study of the link between disease and genetic variation. As a result, genomic data for humans is increasingly available and important in drug development. Increased understanding of the human genome provides insight into the underlying mechanism/s of disease, which in turn supports the development of new approaches to treating and/or preventing diseases [10–12]. To illustrate this link, it is necessary for us to briefly discuss the role of genes in human disease and the effects of xenobiotics on genes. Human diseases are monogenic, chromosomal, or multifactorial in origin: monogenic diseases are caused by changes to a single gene [13, 14], chromosomal diseases are produced by changes in chromosomes [15], and multifactorial diseases are the most common and are caused by variation in many genes, and may be influenced by the environment. Genes are either constitutive or inducible. Constitutive genes are expressed continuously and control the ability of DNA to replicate, express, and repair itself, plus they control protein synthesis and are central to regulating metabolism. In contrast, inducible genes are only expressed intermittently [16]. During the process of gene expression, DNA is transcribed to mRNA, which in turn is translated to protein. Central to the regulation of gene expression is chromatin, a histone-DNA complex. For any given gene, the histone-DNA complex is the inactive state of the gene. One mechanism by which genes are silenced is linked to the presence of positively charged amino acids in histones, which produce zones in the histone–DNA complex that are susceptible to DNA methylation which then regulates gene expression [17, 18]. Small noncoding RNAs, for example, RNAi, may also be involved in the gene regulatory processes. This complex process requires the coordination of modifications to histones, transcription factor binding, and chromatin remodeling and results in the unwinding of the DNA in the transcription zone. As a result, the DNA is accessible to activating and repressor transcription factors (TFs), which bind to a specific DNA-binding domain and an effector domain. On binding an activating TF, the effector domain then recruits RNA polymerase II, allowing transcription of the corresponding gene/s to occur [19–21]. TFs can also activate genes by binding to the enhancer regions, which are located upstream, downstream, or in the introns of a gene. Small noncoding RNAs are also involved in controlling gene expression. Because the regulation of genes involves the interaction of a number of different regulatory cascades, by interfering with these cascades xenobiotics
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The genome is comprised of all the genes, regulatory sequences and noncoding regions of an organism’s DNA. The regulation of gene expression involves the interaction of a number of different regulatory cascades. By interfering with these cascades xenobiotics may alter gene expression, protein/enzyme production and in consequence cellular metabolism. These effects can be monitored by analysis of tissue DNA/RNA profile (transcriptomics), protein/enzyme profiles (proteomics) and metabolite production (metabonomics) (see Fig. 1.1). Analysis of the “-omic” changes in different tissues, resulting from treating animals with a test compound may provide an early, specific indicator of toxicity [22, 23] and help to identify biomarkers of toxicity [24, 25]. This approach has great promise for developing new, specific, sensitive techniques to better characterize, and understand, the nonclinical toxicity of drug development candidates and their risk–benefit ratio. Nevertheless, despite the great strides that have been made in developing and applying these new technologies, the backbone of nonclinical safety assessment remains animal toxicity studies for the time being.

OBJECTIVES OF THIS BOOK

There is a wide range of excellent textbooks available, which review in detail individual and specialist aspects of pharmaceutical toxicology. Our focus is a more broad-based
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and general description of the subject. We describe, with references to key source materials, the background to, and conduct of, the principal nonclinical studies that are central to nonclinical drug development. Although the discussion is primarily based on a description of the development of the low-molecular-weight organic molecules, which have been traditionally developed as pharmaceuticals, the general process we describe is also applicable to newer drug technologies (proteins, nucleic acids, nanoparticles, and the like) linked to recent advances in biotechnology. As we emphasize in individual chapters, regardless of the source and type of test compound or route of administration, the basic toxicological questions to be asked are the same. What changes is the range of studies deployed to answer these questions. What are the relevant questions? They are questions that help:

- the drug development scientist to understand the toxicological profile of the test compound,
- the drug discovery scientist to refine the chemical motif of the test compound to optimize efficacy and reduce side effects and
- the drug development team to advance the test compound to the clinic and then to the marketplace and the patient.

In many instances, the understanding of a complex process, such as drug development, is helped by reviewing real-life cases. This presents a problem, as drug development is done case by case, but, as we show, a baseline approach is provided by regulatory guidelines. We encourage the reader to review the advice we give in this book in the light of the type of compound that they are developing and the drug development strategies deployed for drugs that are currently on the market. While our reference point is the role and conduct of nonclinical studies in the support of drug development, for the most part the subject matter we cover applies more broadly to the toxicological evaluation of chemicals. To illustrate this, we can consider the role of toxicology in the REACH (Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals) process, which was implemented in the European Union (EU) in 2007. The REACH legislation was enacted as a way of managing the risks that chemicals may pose to health and the environment. This legislation applies to chemicals used in industrial processes, cleaning products, paints, clothes, furniture, and electrical appliances. In short, the use of all chemicals in the EU is covered by REACH [26]. In order to meet their legal obligations under REACH, manufacturers and importers of chemicals must identify and manage risks linked to the substances they manufacture and market. To do this, they submit a Registration Dossier to the European Chemicals Agency [27]. One element of this dossier is a Chemical Safety Report (CSR), which describes the chemical safety assessment for the chemical under consideration. In the CSR, registrants must present and discuss a range of data [28]:

- substance identity
- physicochemical properties
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- exposure/uses/occurrence and applications
- mammalian toxicity
- toxicokinetics
- chemical categories
- ecotoxicity
- environmental fate, including chemical and biotic degradation

As this list shows, some data (items highlighted in italics) are similar to the nonclinical data required in drug development. Indeed, if it is available, nonclinical toxicity data can be used. Thus, Chapters 4–10 in this book, which deal with study conduct, types of study, and reporting, also apply to generating toxicity data for the CSR. However, remember that the underlying philosophy differs and thus will alter the risk assessment process relative to pharmaceuticals. In Chapter 11, we discuss the risk management of potential drug toxicities in humans. Again, the general principles that we discuss apply to the REACH risk management process, with the added complication that REACH also requires the preparation of an environmental risk management plan, which falls outside the scope of this book. Our attention is mostly on the “scientific” aspects of nonclinical toxicology. However, Chapters 3 and 4, and, to some extent, Chapter 10, deal with administrative/organizational aspects of nonclinical studies. These activities are sometimes overlooked, or relegated to a secondary importance; this is a mistake. Time spent optimizing these aspects of nonclinical activities can produce significant savings in terms of time and resources and reduces the possibility of errors in study conduct, data interpretation, data reporting, and risk management.

REFERENCES

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