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Molecular and Biochemical Toxicology: Definition and Scope

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1.1 Introduction

Since the previous edition, toxicology has seen a dramatic increase in the application of the principles and methods of molecular approaches, particularly as it relates to targeted and unbiased approaches in transcriptomics, epigenomics, proteomics, and metabolomics. Biochemical and molecular toxicology is concerned with the definition, at the molecular and cellular levels, of the cascade of events that is initiated by exposure to a toxicant and culminates in the expression of a toxic endpoint. Molecular techniques have provided a wealth of mechanistic information about the role of gene function in the interaction of xenobiotics and living organisms. The development of knockout and knockin mice, as well as "humanized mice" with human genes inserted into their genome, and collaborative cross mice with genetic diversity similar to human populations has proven extremely valuable in investigations of toxicant metabolism, modes of toxic action, and gene-by-environment (GxE) interactions. Diverse models including zebrafish, *C. elegans*, yeast, *Drosophila*, and *Daphnia*, among others, also provide important models to study the molecular mechanisms of how environmental toxicants promote disease and induce toxic effects. The field is making rapid and significant progress in understanding the complex molecular and biochemical mechanisms through which environmental stressors interface with pathways, the genome, and the epigenome to influence toxicity and health outcomes, and these advances are reflected in this edition, the fifth. From the biomolecule, pathway, cell, tissue, organ, model

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organism, and human-to-human population, the new edition integrates these levels of biological organization.

Toxicology can be defined as the branch of science dealing with poisons. Having said that, attempts to define all of the various parameters lead to difficulties. The first difficulty is seen in the definition of a poison. Broadly speaking, a poison is any substance causing harmful effects in an organism to which it is administered, either deliberately or by accident. Clearly, this effect is dose related inasmuch as any substance, at a low enough dose, is without effect, while many, if not most, substances have deleterious effects at some higher dose. Much of toxicology deals with compounds exogenous to the normal metabolism of the organism, such compounds being referred to as xenobiotics. However, many endogenous compounds, including metabolic intermediates such as glutamate or hormones such as thyroxine, are toxic when administered in unnaturally high doses. Similarly, trace nutrients such as selenium, which are essential in the diet at low concentrations, are frequently toxic at higher levels. The production of reactive oxygen species (ROS) and subsequent events is a frequent sequel to interaction with xenobiotics that has significant consequences in terms of cell toxicity and health outcomes.

The expression of toxicity and the assessment of toxic effects are other parameters of considerable complexity. *Acute toxicity*, usually measured as mortality and expressed as the lethal dose or concentration required to kill 50% of an exposed population under defined conditions (LD50 or LC50), is probably the simplest measure of toxicity. Nevertheless, it varies with age, gender, diet, the physiological condition of the animals, environmental conditions, and the method of administration. *Chronic toxicity* may be manifested in a variety of ways, including cancer, cataracts, peptic ulcers, and reproductive effects, to name only a few. Furthermore, chemicals may have different effects at different doses. For example, vinyl chloride is a potent hepatotoxicant at high doses and a carcinogen with a very long latent period at low doses. Considerable variation also exists in the toxic effects of the same chemical administered to different animal species or even to the same animal when administered via different routes. Malathion, for example, has relatively low toxicity to mammals but is toxic enough to insects to be a widely used commercial insecticide.

Additionally, there are critical windows of susceptibility to certain toxicants; for example, exposures to certain toxicant or dietary conditions during development can influence later susceptibility to certain adult diseases. As described in "Developmental Origins of Health and Disease" (Chapter 28), exposures to certain toxicants or dietary conditions during development can modify the epigenome, and these modifications are important determinants of certain adult diseases/adverse health outcomes.

1.2 Sources of Information

In keeping with the textbook format, the most important sources of information are appended to all 29 chapters as "Suggested Reading" for the user. Taken together they form an in-depth source of extended information on all aspects of molecular and biochemical toxicology and serve to promote further understanding. Having individual chapter "Suggested Reading" lists rather than a consolidated list is more user friendly and, more important, facilitates a more focused exploration of specific topics. The current fifth edition consists of 29 chapters and is divided into 5 areas following each other in logical sequence. They are:

1) Introduction
2) Techniques in Molecular and Biochemical Toxicology
3) Mechanisms in Molecular and Biochemical Toxicology
4) Molecular and Biochemical Aspects of Organ Toxicity
5) Emerging Areas in Molecular and Biochemical Toxicology

1.3 Toxicology

Toxicology is clearly related to two of the applied biologies: medicine and agriculture. In medicine, clinical diagnosis and treatment of poisoning as well as the management of toxic side effects of clinical drugs are areas of significance. In agriculture, the development of selective biocides such as insecticides, herbicides, and fungicides is important, and their nontarget effects are of considerable public health significance. Toxicology may also be considered an area of fundamental science because the adaptation of organisms to toxic environments has important implications for ecology and evolution. Toxicology is a critical part of environmental health science and occupational safety and is important in understanding individual and population susceptibility.

The tools of chemistry, biochemistry, and molecular biology are the primary tools of toxicology, and progress in toxicology is closely linked to the development of new methodology in these sciences. Those of chemistry provide analytical methods for toxicants and their metabolites, particularly for forensic toxicology, residue analysis, and toxicant metabolism as well as for proteomic and metabolomics to measure changes in proteins or metabolites, respectively, such as biomarkers of exposure or disease or related to the mechanism of toxicity. Methodologies of biochemistry are employed for the investigation of metabolism and modes of toxic action and those of molecular biology for investigations of the roles of genes and gene expression in toxicity and GxE interactions.

Molecular and biochemical toxicology deals with processes that occur at the cellular and molecular levels when toxic chemicals interact with living organisms. Defining these interactions is fundamental to our understanding of toxic effects, both acute and chronic, and is essential for the development of new therapies, for the determination of environmental occupational toxic hazards, and for the development of new clinical drugs for medicine and biocides for agriculture.

The poisoning process may be thought of as a cascade of more or less distinct events. While biochemical and molecular toxicology is involved in all of these, their involvement in exposure analysis is restricted to the discovery and use of biomarkers of exposure. Following exposure, uptake involves the biochemistry of cell membranes and distribution or transport processes within the body (see Chapter 11). Metabolism, which may take place at portals of entry or, following distribution, in other organs, particularly the liver, may either detoxify toxicants or activate them to reactive metabolites more toxic than the parent chemical (see Chapter 8, "Phase I and Phase II Metabolism and Metabolic Interactions: A Summary"). Polymorphisms in genes that metabolize xenobiotics have important toxicological consequences as described in "Polymorphisms in Phase I and Phase II Genes and Outcomes" (Chapter 10). Chemicals with intrinsic toxicity or reactive metabolites are involved in various modes of toxic action, usually initiated by interactions with macromolecules such as proteins and DNA. Chapters on DNA damage and mutagenesis (Chapter 16), DNA repair (Chapter 17), and carcinogenesis (Chapter 18) focus on the outcomes of the interactions of these reactive metabolites or ROS with DNA. ROS produced by endogenous or exogenous molecules are important in toxicity and have been implicated in numerous human diseases. How damaged cells make decisions to live or die is described in "Mechanisms of Cell Death" (Chapter 13), and these decisions have important consequences in diseases such as cancer and neurodegeneration. The study of modes of toxic action is a critically important area of toxicology.

Many of these fundamental molecular mechanisms previously alluded are studied at the organ level (discussed in Chapters 19 through 25), including those responsible for molecular mechanisms of respiratory toxicity (Chapter 19), molecular mechanisms of hepatotoxicity (Chapter 20), molecular mechanisms of renal toxicity (Chapter 21), molecular mechanisms of neurotoxicity (Chapter 22), molecular mechanisms of immunotoxicity (Chapter 23), molecular mechanisms of reproductive toxicity (Chapter 24), and molecular mechanisms of developmental toxicity (Chapter 25).

1.4 Molecular and Cellular Toxicology

The culture of cells isolated from living organisms has been known since the early years of the twentieth century. In the 1950s the development of standardized culture media and the development of immortalized cell lines increased

the utility of cultured cells in many areas of experimental biology, including toxicology. The use of cell culture in toxicological research is an established and useful approach for a number of reasons, including its use in investigating toxic effects on intact cellular systems in a situation less complex than that in the intact organism and its potential utility for routine toxicity testing systems for regulatory evaluations.

Some cells, such as hepatocytes, must be used in primary culture since they will not divide in culture and are relatively short lived, while other cell lines are capable of division and can, in suitable media, be maintained indefinitely. Many cell lines retain the properties of the original cell type *in vivo*. All of the various approaches to the use of cultured cells in biochemical and molecular toxicology are summarized in Chapter 6 ("Cellular Techniques"). The union of the techniques of cell and molecular biology has been enormously productive for experimental toxicology since cells can be used for the expression of genetic constructs, depletion of the gene of interest via siRNA knockdown technology, gene editing via CRISPR/Cas9, fluorescence-activated cell sorting analysis, and so on.

The field of molecular biology is usually held to have begun with the description of the double helical structure of DNA by Watson and Crick in 1953, followed by the elucidation of the genetic code in the 1960s. In the subsequent half century, the techniques of molecular biology have expanded exponentially as has its importance in many, if not most, fields of biology. The success of the human genome project has given rise to an entire field devoted to the description of the complete genomes of organisms at all levels in the evolutionary tree. An overview of molecular techniques used to study gene function and transcription regulation as well as proteins and small molecules are presented in Chapter 2 ("Molecular Techniques for the Study of Gene Function") along with Chapter 3 ("Trancriptomics"), Chapter 4 ("Proteomics"), and Chapter 5 ("Metabolomics"). In addition, to the importance of classical genetic and genomics to molecular toxicology, epigenetics is emerging and an important area. Epigenetics is defined as chemical changes to DNA that do not alter the genetic sequence, such as the methylation of cytosine residues that influence the recruitment of protein such as transcription factors to DNA. The consequence of epigenetic changes including cytosine methylation and histone modification is the tight regulation of gene expression. DNA methylation, along with other epigenetic modifications, explains how cells within the human body with the same genetic complement are able to differentially express genes to enable them to perform distinct functions. An individual's epigenome is malleable and influenced by environment toxicants/agents, and these environmentally induced changes can contribute to disease susceptibility.

The techniques that have proven most valuable in toxicology include those of molecular cloning, the polymerase chain reaction, and the production of genetically modified mice. Microarrays and, more recently, RNA sequencing

(RNA-seq) are used to evaluate gene expression under various conditions, including exposure to toxicants, which are becoming more important, the latter permitting a global approach to gene expression across the entire genome. These in concert with other molecular techniques are being considered as potentially useful tools in such applied areas as hazard assessment and risk analysis.

1.5 Proteomics and Metabolomics

Since molecular biology is often held to be restricted to events involving nucleic acids, mention must be made of proteomics, the analysis of all proteins or subset of proteins in a sample of biological material, and metabolomics, the analysis of all or a subset of metabolites in a sample of biological material. Proteomics is key methodology in toxicology as it is the proteins that produce the cellular phenotype and regulate cellular function and structure. Analysis of the proteome and how it can be perturbed by toxicants is highly informative and can reveal changes in cellular pathway and networks (Chapter 4). Targeted proteomics often examines posttranslational modifications of the proteins of interest or protein–protein, protein–DNA, or protein–RNA interactions, sometimes referred to as the protein interactome. Metabolomics involves the analysis of the low molecular weight complement of cells, tissues, or biological fluids, focusing on the detection of compounds with less than 2000 molecular weight (Chapter 5). For metabolomic studies, analytical and mathematical approaches are used to determine signals that increase or decrease in tissues and biological fluids in studies designed to assess the impact of exposure (e.g., drugs, chemicals, stress) and/or health status. While specific genes can be identified that define individuals' risks for a disease or response to treatment, metabolites inform us about the state of a disease, dysfunction, or disorder at the time of sampling. The study of metabolites can reveal how exposure impacts the biochemistry of an individual and provide biomarkers related to exposure and health outcomes. These fields are discussed in Chapters 4 ("Proteomics") and 5 ("Metabolomics").

1.6 Role of Molecular, Cellular, and Biochemical Toxicology: Implications for Risk Assessment

Since the publication by the US National Research Council of the very influential "Toxicity Testing in the 21st Century: A Vision and Strategy," considerable efforts have gone into implanting their forward-looking precepts. They called for high-throughput methods utilizing human or human-derived cell lines, integration of high- throughput screens (HTS), and cell systems.

Computational toxicology is a key area in addition to the molecular aspects (Chapter 26). Since the number of chemicals is high, too high in fact for traditional risk assessment, these methods have a cellular, biochemical, and/or molecular basis. They include HTS to assess various aspects of cytotoxicity, cell responses, gene expression, and activation of specific signaling pathways and transcription factors. Much the same ideas can be found within the REACH legislation of the EU. The leading US governmental agency in these endeavors is the US Environmental Protection Agency with other agencies, particularly National Institute of Environmental Health Sciences.

It should be emphasized that none of these methods have yet been adopted for regulatory purposes but that, in the future, they almost certainly will be, making this a fertile field for those in applied molecular toxicology now and in the future.

1.7 Conclusions

The preceding brief description of the nature and scope of biochemical and molecular toxicology and their use in many aspects of toxicological science should make clear that the study of toxic action is multifaceted, covering all aspects from the initial environmental contact with a toxicant to its toxic endpoints and to its ultimate excretion back into the environment. Biochemical and, more recently, molecular toxicology forms the mechanistic basis for all of these aspects.

Although a considerable amount of material is summarized in the following chapters, many essential aspects and their applications still remain to be discovered.

Suggested Reading

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