

PART I

INTRODUCTION TO TOXICOLOGY METHODS

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AN INTRODUCTION TO TOXICOLOGY AND ITS METHODOLOGIES

ALAN B. COMBS AND DANIEL ACOSTA JR.

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1.1 OVERVIEW

Toxicology in the broadest sense is the study of the adverse effects of drugs or chemicals on living systems. The questions asked by this discipline include what things are toxic, how and why toxicity is manifested, and how might toxicity be predicted, treated, or prevented. It is the purpose of this chapter to give a broad introduction to toxicology and to show how modern computational techniques are becoming so useful to the field.

1.2 WHERE AND WHY TOXICOLOGICAL KNOWLEDGE IS IMPORTANT

Our modern industrial society is highly dependent on chemical entities for its very existence. Useful chemicals cover the gamut from the building materials that make up our dwellings and machines, to the fertilizers and pesticides used in our production of food, to the chemicals used in our manufacture of electronics and communications. These chemicals include the drugs and materials used in medicine and health care. Many new biologically active and useful compounds result from the activity of our pharmaceutical industry in areas of biotechnology. The production of each of these materials leads to industrial waste and the potential for environmental pollution. Because we become exposed to all of these things, prudence and regulations dictate that their potentials for toxic risk must be determined. Toxicologists are involved in all facets of this risk evaluation. The purpose of this chapter is to introduce the endeavors used to evaluate risk in the field of toxicology and to indicate why instrumentation and computation are necessary.

1.3 INDISPENSABLE DISCIPLINES FOR THE SCIENCE OF TOXICOLOGY

It has long been a matter of honor and pride that pharmacologists and toxicologists must be highly conversant with so many different sciences. The

disciplines needed for toxicology include many of the life sciences, mainly biology, zoology, botany, physiology, genetics, pharmacology, biochemistry, histology, and pathology. Analytically related methodologies used in toxicology include analytical chemistry, flow cytometry [1], the techniques and tools of modern genetics, and molecular biology. Statistics is involved in study design, data analysis, and interpretation. In effect, the topic of this book, the use of *computation* in the gathering of the massive amounts of data generated by modern toxicology, the documentation of these efforts, and the interpretation of the resulting data have become more and more essential and increasingly routine in toxicology.

Biology, zoology, and physiology predict the normal responses of living systems, whose deviations can help define the effects of toxic substances on these systems. Toxic effects may produce adverse changes at the biochemical, tissue, organ, and organism levels. Again, perturbations from normal function or anatomy can help define toxic effects. Histology is the study of normal microanatomy, and pathology describes what happens to these microstructures when they become injured by toxicants. Many different sophisticated analytical techniques are used in the most advanced studies.

1.4 SUBDISCIPLINES OF TOXICOLOGY

In its role of explaining, predicting, preventing, and treating the adverse effects of drugs and chemicals, toxicologists are working in many subdisciplines. They are involved in drug and chemical *safety screening* and in their *regulatory* counterparts, the EPA, FDA, and USDA in the United States. They are involved in occupational and industrial toxicology and in their own particular scientific and *regulatory* counterparts, NIOSH and OSHA. In addition there are people specializing in *forensic*, *veterinary*, and *clinical* toxicology. Finally there are scientists involved in mechanistic studies at all levels of the organism's organization.

The whole idea behind toxicological testing and safety screening is the potential benefit to humans and animals that will accrue. This entails defining the risk of exposure to drugs and chemicals, understanding the risk when it exists, and preventing the risk. This concept holds whether one is doing drug discovery, environmental, or regulatory toxicology.

As described, toxicologists in chemical and pharmaceutical industries work to define the risk associated with new drugs and chemicals. Such safety evaluation is part of the art and science of toxicology, though much of the methodology is codified in the law. Regulatory toxicologists acting for the general public welfare work to create and ensure adherence to safety regulations. The process of discovery is part collegial and part adversarial as investigators and regulators strive to fill their co-dependent function.

Forensic toxicology combines analytical chemistry, knowledge of toxicology, and detective work to determine the causes of those cases of poisoning

that have become of interest to law enforcement or regulatory agencies. Veterinary toxicology and human clinical toxicology deal with the evaluation and treatment of poisoning.

1.5 TRADITIONAL TOOLS OF TOXICOLOGY

Epistemology is the undertaking of how we know what we know, or the study of knowledge and the basis of its validity. Toward this end in toxicology we bring all the tools of our science and our rationality. This entails the appropriate gathering of information and its proper evaluation and interpretation.

Properly designed and interpreted animal studies are the primary tools of safety screening and predictive toxicology. Among the basic techniques used by toxicologists are dose–response studies. Articulated first by Paracelsus [2] is the idea that it is the dose that makes the poison. All things are dangerous in large enough doses and all things are safe if exposure is small enough. Additionally the demonstration of a dose–response relationship between a tested substance and the effect it is suspected to produce provides strong evidence that a cause–effect relationship exists between them. As the basis for regulatory toxicology, the existence of a *threshold dose*, the dose below which no adverse effect occurs, provides the basis for recommended maximal exposures that are safe.

Many of the fields of study and types of toxicology are described below. These efforts are very broad and entail the use of many disciplines.

1.6 FIELDS OF EXPERTISE WITHIN TOXICOLOGY

Toxicology can be classified according to the effects on the organ systems damaged. Alternatively, it can be classified according to the mechanisms of toxicity. Almost anything that can go wrong with almost any tissue in the body will occur. Each of these areas comprises its own realm of toxicological expertise. We will first examine several examples of organ-based toxicity. In some cases there will be extensive overlap between categories. One of the important questions is why there frequently is specific target organ toxicity. We will examine some aspects of this question. Most frequently the answer relates to the specific biological characteristics of the tissues.

1.6.1 Chemical Carcinogenesis

Because of the intense public interest that exists in cancer prevention and the resulting political interest, chemical carcinogenesis is a gigantic and relatively well-funded field. Amazon.com (as of June 2006) lists nearly 80 books in print on the topic. Google.com lists over 200,000 hits on the topic. The National Library of Medicine’s Medline lists nearly the same number of articles on the

topic within the scientific or medical literature. Not only is the extent of effort indicated by these numbers, but also the diffuse, difficult, intractable, and fractal nature of the field.

Carcinogenesis is a multiple-step, progressive process. Causes of such damage can include alkylating agents, active oxygen species, and radiation. These causes of injury can also lead to genetic toxicology. Much of the current mechanistic interest is centered on dysregulation of cellular growth control mechanisms [3].

1.6.2 Genetic Toxicology

There is a great degree of overlap between the topics of genetic toxicity and chemical carcinogenesis. This is because many of the stepwise changes that occur during the development of neoplasia consist of somatic mutations that result in changes in growth regulation of the affected tissues.

The field more commonly thought of as *genetic toxicology* deals with changes in what might be termed *legacy genes*, those genes that are passed from one generation to the next. These changes occur as a result of unrepaired injury to the cellular DNA and the effects are almost always bad. The safety screening required for carcinogenesis in drug and chemical discovery is extensive [4].

1.6.3 Developmental Toxicology/Reproductive Toxicology

The topic of teratogenesis as a disruption of the control of embryological development is covered later in this chapter. Safety screening requires evaluation of developmental and reproductive toxicity of the compounds of interest [5].

1.6.4 Blood and Bone Marrow

Hematotoxicity is another area of active investigation. Benzene is an excellent example of the extremes that can be caused by substances that are toxic to the bone marrow. Chronic exposure to benzene can cause either leukemia, or bone marrow injury that can lead to aplastic anemia [6,7]. Agranulocytosis and aplastic anemia are infrequent but deadly toxic effects of several drugs. Anemias related to deficiencies of each of the formed elements of the blood also are known, and some of these are toxicological in origin. For example, thrombocytopenia is an established and potentially deadly adverse effect of heparin, though the etiology may be immunological [8].

1.6.5 The Immune System

The function of the immune system is to protect the internal environment of the body against external attack [9]. Because the nature of the attack can be

so varied, bacterial, fungal, viral, and the presence of foreign proteins, the immune system has become one of great complexity. Antibody-mediated immunity and cellularly mediated immunity both exist, and the stimulus–response characteristics of this system and the necessary control mechanisms are also very complex.

Decreased immunological competency can lead to susceptibility to infections, and it can also lead to cells that lack the capacity to control their growth. In contrast, excess activity can cause the immune system to attack the host organism, itself. Both of these adverse effects can result from xenobiotic exposure.

Among the drugs that can decrease immunological competence are anti-inflammatory steroids, cyclosporine, and tacrolimus. Certain of these compounds are used to prevent transplant rejection, but they simultaneously carry the risk of allowing infection to occur. The aplastic anemia caused by the bone marrow toxicity of benzene was described above. Lead and chlorinated aryl hydrocarbons such as hexachlorobenzene also can cause bone marrow suppression.

Inappropriate immunological activation has been known for a long time. Anaphylaxis following sensitization is an example. Another example is that untreated beta-hemolytic streptococcus infections can lead to rheumatic fever and damage to the heart valves. This appears to occur because the streptococcus organism and our heart valves share a common antigen, and development of immunity against the former leads to damage to the latter.

Immunotoxicology is a discipline still in its infancy. Perhaps, this is most clearly bourn out by the recent experience in Great Britain in which a monoclonal antibody that was designed as an agonist to a receptor on T-lymphocytes was first given to six human volunteers. The dose given was much lower (500 times) than that which had been safe in animals. Nevertheless, the result was a massive release of cytokines leading to global organ failure. At this time all have survived the event, though it was not certain for some time that this would happen. This is an excellent example of species differences, and it is clear that much more work must be done to characterize human immunological responses when potential immunological stimulants are in the process of drug discovery [10,11].

1.6.6 The Liver

The liver has two main functions in the body [12]. The first is maintenance of internal nutritional homeostasis through facilitation of lipid absorption and intermediary metabolism. As described later, the large metabolic capacity of the liver renders it vulnerable to heavy metals through binding of the metals to and inactivation of electrophilic ligands.

The second function of the liver is to deal with various endogenous substrates and dietary xenobiotics through their metabolism and biliary excretion. Several toxicities are associated with disturbances of this function. One

example is the oxidative dechlorination of carbon tetrachloride and other chlorinated hydrocarbons to free radical metabolites that bind to and destroy hepatic tissues. Another example of toxicity by metabolic activation occurs with acetaminophen. A trace metabolite of acetaminophen is a very reactive quinoneimine. Under normal usage of this analgesic, this metabolite is not a problem because it is inactivated by binding to reduced glutathione. However, when an overdose of acetaminophen is taken, the protective glutathione becomes depleted and the reactive metabolite covalently binds to and destroys hepatic parenchymal tissue.

Cirrhosis of the liver is one of the most well-known adverse effects of chronic alcohol abuse. The cholesterol-lowering, life-prolonging statin drugs must be monitored routinely for hepatotoxicity and rhabdomyolysis. A Google search on the terms “statins,” “hepatotoxicity,” and “review” produced over 22,000 hits indicating this is a very active field of interest.

1.6.7 The Kidney

For the same reasons as described for the liver, heavy metals and compounds converted to active metabolites can also be toxic to the kidney, which is very active metabolically [13,14]. With certain quinones, reduced glutathione can enhance toxicity, rather than being protective [15].

Gentamycin and other aminoglycoside antibiotics are toxic to the kidney. Use of these compounds necessitates repeated dosage adjustments according to drug blood levels.

1.6.8 The Respiratory System

As is the case with the skin, the lungs are in constant contact with the external environment [16]. Exposure to the toxins in cigarette smoke is one of the most common causes of congestive, obstructive damage in the respiratory system. Occupational exposure to asbestos and medically necessary exposure to drugs such as cyclophosphamide and carmustine can also cause lung injury. Inhalations of coal dust and cotton fibers are other occupational hazards to the lungs.

1.6.9 The Nervous System

The central nervous system is one of the most complex organs in living systems [17]. Neurotoxicity can be manifested rather globally, or very specifically, depending on the poison. One example of a very specific toxicity occurs with MPTP, a notorious meperidine analogue that can destroy the substantia nigra and leads to a very severe Parkinson-like syndrome. Another example of rather global neurotoxicity occurs with lead encephalopathy. Other metals can also be highly neurotoxic.

Developmental retardation occurs following exposure to metals, and this has been instrumental in decreasing the amount of lead used in gasoline and indoor paints. Maternal alcohol drinking during pregnancy can also cause developmental retardation manifested as fetal alcohol syndrome. Organophosphates can cause acute injury related to acetylcholine accumulation and certain ones such as triethocresylphosphate can cause delayed axonal degeneration. Picrotoxin, camphor, and strychnine are examples of powerful convulsants. Anesthetics and analgesics can lead to respiratory depression and hypoxia. Carbon monoxide and cyanide also cause general hypoxic damage to the brain and to other high oxygen demand organs of the body. The literature abounds with other examples of substances that are toxic to the nervous system.

1.6.10 Behavioral Toxicity

Many poisons can disturb mental and rational function leading to behavioral abnormalities. Psychototoxins include phencyclidine, LSD, and fungal toxins. Less commonly, stimulants such as cocaine and amphetamine can cause psychiatric problems. Psychiatric effects of high doses of corticosteroids have also been described. In addition to the developmental retardation, some investigators believe that cognitive impairment, hyperactivity, and perhaps even anti-social behavior may be caused by childhood lead exposure. Public discussion of these subtle toxic effects is highly politicized because childhood exposure to lead still occurs as a risk factor in slums and tenements.

1.6.11 Cardiotoxicity

Compared to many of the other organs, the heart must continuously maintain beating activity [18]. There is little energy storage capacity in the heart, which therefore must be producing the energy it uses in real time. Drugs that decrease the capacity of the heart to use substrate and generate ATP can be very harmful to the heart. Examples of toxicants believed to act by this mechanism include cyanide, glycolysis inhibitors such as emetine [19], and Krebs-cycle metabolism inhibitors such as the cardiotoxic anthracycline doxorubicin [18].

In addition to the necessity of continuous energy generation, the heart must maintain rhythmic function throughout its lifetime. Substances such as cocaine and cyclopropane that decrease the reuptake of norepinephrine after its release from noradrenergic neurons are prone to cause fatal arrhythmias. Additionally drugs that modify plasma membrane ion channel function can also cause arrhythmias. More recently cardiotoxicity from drugs that prolong the QT-interval has been reported. Such drugs include several anti-microbial agents, antidepressants, and anti-migraine agents. This broadly based toxicological effect has clear implications for the drug discovery process [20].

1.6.12 Dermal Toxicity

The skin is the primary organ of contact between the organism and its environment. There is extensive commercial interest in dermal toxicology and safety screening because of the many different products used topically for therapeutic and cosmetic purposes. Similar comments can be made about ocular products.

Some of the toxic effects to the skin are allergic in nature. The response to poison oak or poison ivy is an example. Corrosive injury to the skin can occur following contact with many household products. Cutaneous responses to certain drugs can include dangerous exfoliative dermatitis and the Stevens-Johnson syndrome [21,22].

1.6.13 The Reproductive Systems

In addition to chemical carcinogenesis, teratogenesis is a toxic effect that catches the public's attention. The public response to thalidomide was so great that it is still very difficult to get the drug approved for newer indications. Once we know what a toxic effect can be, toxicologists are quite effective in developing animal tests that screen for that effect. For example, the fetotoxic effects of compounds such as Accutane® and the angiotensin converting enzyme inhibitors are known from screening studies, and a large teratogenic disaster such as thalidomide should not happen, again. It is a commentary on human nature that the fetal alcohol syndrome still continues to occur.

1.6.14 Endocrine Systems

Toxic changes can be caused by endocrine agonists, antagonists, and disruptors. There are estrogen active compounds such as diethylstilbesterol and dioxin. Natural and synthetic thyroid antagonists such as propylthiouracil are known. Agonists and antagonists for adrenocortical hormones have been described. Oral contraceptives are risk factors for increased blood clotting and stroke. Estrogens are risk factors for breast and uterine cancers, and there is much interest in the associated risks from environmental estrogen pollutants (e.g., the REACH initiative).

1.7 IN VITRO METHODOLOGIES FOR FIELDS OF EXPERTISE WITHIN TOXICOLOGY

Biomedical and toxicological research and safety screening require the use of animals [23]. However, since the inception of the first animal welfare organizations, society's use of animals has been a matter of concern and controversy [24]. Because of this interest there has been much activity in the past few decades in finding alternative methods for doing research and screening. The

“Three Rs” of Russell and Burch [25], *replacement*, *reduction*, and *refinement*, have been the goals of much of this work. Many alternatives to animals have been suggested, and where the alternatives have been verified to be useful, it is appropriate that they be used. One of the best examples of replacement is the current use of *Limulus* (horseshoe crab) serum to detect the presence of the gram-negative organism endotoxins known as pyrogens [26]. Parenteral products must be sterile and pyrogen-free. *Limulus* serum is more sensitive to the presence of these harmful proteins than the rabbits that were previously used. Not many other alternatives have been so well verified, however.

The current all out attack mounted by animal activists on the societal use of animals is a matter of extreme concern to toxicology and the other biomedical sciences. Some of the best resources to inform ourselves and to counter these activists are the frequently asked questions (FAQ) detailed in the Animal Rights Myths FAQ [27].

Cell culture is one of the primary methods being studied for animal replacement. Primary cultures of heart cells, liver, keratinocytes, corneal cells, and many other tissues are actively being studied [28]. Much work has been done for decades in some cases to maintain the functions of the parent tissue close to those in vivo while the cells are in culture. Eventually most differentiated function of the cells is lost. The art is to maintain such function for as long as possible so that longer in vitro exposures can mimic in vivo dosing.

Propagated cell lines are also widely used. Such cells are immortalized by combination with neoplastic cells. One problem with these cell lines is that they frequently do not express any of the differentiated functions of the parent cells, and therefore do not provide tissue specific responses.

One of the failings of cell culture in predictive toxicology is that some examples of toxicity are multi-organ in nature. Methanol toxicity, for example, occurs when the methanol is oxidized in the liver to formate. The formate is transported by the blood to the retina and CNS where it produces its characteristic effects of blindness and brain damage. To model methanol toxicity in cell culture would require co-cultures of liver and retinal cells. Co-cultures are technically difficult, and it would be very difficult to predict which multiple cell types are needed in a co-culture to detect a previously unknown toxicity. It requires an intact organism to do this.

Innumerable cell lines are used in studies trying to understand intracellular messengers and control processes. Such models are particularly useful provided that the cells remain viable as almost any desired genetic alteration can be produced and studied.

1.8 MECHANISMS OF TOXIC INJURY

Although many different cells and tissues can be injured by toxicants, there are not many different fundamental mechanisms by which injury can occur. Each of these categories can be very broad, however. Mechanisms of injury include *ligand binding* by heavy metals, *covalent binding*, *oxidative stress* by

active oxygen species, *antimetabolites*, the *extension of pharmacologic action*, *dysregulation of cellular signaling*, and a miscellaneous category, all of which are now described in a little more detail.

1.8.1 Ligand Binding by Heavy Metals

Electrophilic ligands such as sulfhydryls, amino groups, and hydroxyl groups are found at the active sites of many, if not most enzymes [29]. These ligands have a high affinity for many different metals (e.g., Hg, As, Pb, Sb), and the uptake of and binding of metals to these sites inactivates them. The effect that occurs depends on the location and function of the enzymes in the tissue involved. The more metabolically active a tissue is, the more it is likely to be adversely affected by metals. The liver, kidneys, gastrointestinal mucosa, and central nervous system are particularly vulnerable because they are so metabolically active. Teratology of the toxic metals is also an issue. The major differences between the metals are more a matter of pharmacokinetics than fundamental differences in mechanisms of toxicity.

The antidotes for heavy metals are called chelating agents, a picturesque term invoking an image of lobster claws (chela) grabbing hold of the metal. Such drugs are rich sources of the ligands to which metals readily bind, and these drugs are able to compete effectively for the metal against the endogenous tissue ligands.

1.8.2 Covalent Binding to Biological Macromolecules

This toxic mechanism, which had its most active interest in the late 1970s and early 1980s, occurs when chemicals are metabolized to free radicals or other highly active molecules. These radicals then covalently alkylate nearby macromolecules. Such macromolecules can include proteins, cellular membranes (including plasma membranes, nuclear membranes, membranes of organelles, etc.), and genetic components such as DNA and RNA. If metabolically critical areas of these large molecules become covalently bound to a metabolic product, they may become inactivated. Specific toxic effects will depend on which biological macromolecules become inactivated. An example is the case of carbon tetrachloride that becomes oxidized by liver P450 enzymes to the trichloromethane free radical. This active alkylating agent attaches itself to nearby liver parenchyma, resulting in the classic liver toxicity described for carbon tetrachloride. Research interest in covalent binding as a mechanism of toxicity has decreased since the 1980s because it is very difficult to determine which of all the structures in the body that become alkylated is ultimately responsible for the toxicity produced.

1.8.3 Oxidative Stress

Oxidative stress is a general term for the excessive production of *active oxygen species* and the resulting biological responses [30]. The various oxygen species

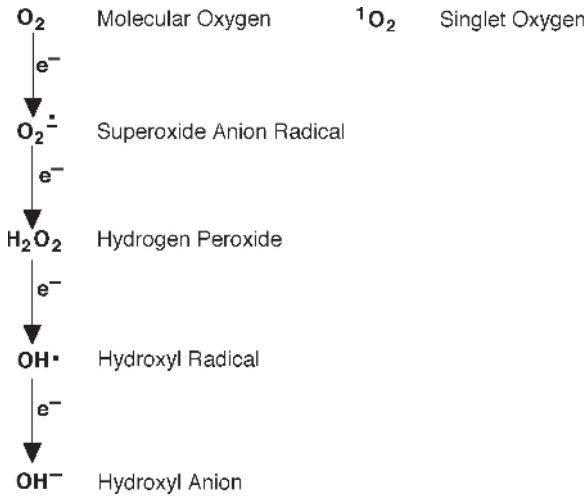


Figure 1.1 Various valence states of oxygen as a function of increasing single-electron reductions.

are depicted in the schematic of Figure 1.1 as a series of one-step electron reductions—starting with molecular oxygen and ending with the hydroxyl anion.

Active oxygen species are usually produced within cells as by-products of normal oxidative metabolism. The location of these processes can include the cytochromes P450, mitochondria, lysosomes, and peroxisomes. As was the case with damage by free radical metabolic remnants leading to covalent binding and injury, several of the active oxygen species can also cause damage to biological macromolecules.

In addition lipid peroxidation can result from action of active oxygen species. This leads to destruction of metabolically necessary lipid molecules and damage to the structural integrity of cellular membranes. Damage from oxidative stress can occur with excessive production of active oxygen species, inadequate protection against such species, or both. Examples of toxicity from active oxygen species include the pancreatic beta-cell destruction by alloxan, the neurotoxicity of 6-hydroxydopamine, the cardiotoxicity of the anthracycline antibiotics, and the pulmonary toxicity of the herbicide paraquat.

Singlet oxygen is a unique form of activated oxygen. It is most commonly involved in phototoxic reactions. Because of absorption of an energetic photon, one of the previously paired electrons of the molecule has been promoted to an orbital of higher energy. In the strictest sense it is not a free radical, but it can act as an active oxygen species. Certain compounds such as tetracyclines or amiodarone can trap photons upon exposure of the skin to the ultraviolet portion of the light spectrum. These photon-activated compounds can pass their energy to molecular oxygen, converting it to singlet oxygen. Singlet

oxygen, in turn, passes its excess energy to dermal tissues, resulting in tissue damage and sunburn.

1.8.4 Antimetabolites

Antimetabolites compete with normal endogenous substrates and cause inhibition of the processes that require those substrates. Examples include purine and pyrimidine antagonists, which prevent nucleic acid replication and cellular division in cancer chemotherapy. Another example is methotrexate, which can inhibit folic acid metabolism.

1.8.5 Denaturing Agents

Denaturing agents can destroy the tertiary structure of proteins. Alcohol's antiseptic action results from denaturing of bacterial proteins. Corrosives can cause tissue damage upon accidental exposure.

1.8.6 Extension of Pharmacology

This is a broad category of toxic action in which exaggeration of the therapeutic effects of many drugs in overdose can lead to poisoning. For example, general anesthetics are also respiratory depressants, and too high concentrations can cause fatalities. Many antihypertensives cause potentially fatal vascular collapse and shock when taken in overdose. Overdoses of certain antiarrhythmic drugs can themselves cause fatal arrhythmias, actions that are related to their action on ion channels.

1.8.7 Dysregulation of Cell Signaling

This is one of the currently most active areas of toxicological research interest, and it is one with many different research thrusts. There is much current interest, for example, in the mechanisms of regulation of apoptosis, programmed cellular death. Production of apoptosis when it should not occur, or its lack when it normally should occur, can each be mechanisms of toxicity. Part of the adverse remodeling of cardiac and vascular tissue can occur because of hypertension-induced apoptosis of cardiac and vascular cells [31]. Apoptotic processes also have been implicated in alcoholic hepatotoxicity [32].

Excessive inflammatory responses may result from inappropriate cellular signaling. On the other hand, inflammation is normally a protective response, and its lack can lead to increased susceptibility to infections. Dysregulation of cellular division can lead to neoplasia or aplasia. Neoplastic changes reflect a dysregulation of cell growth, whether from failure of apoptosis or other mechanisms.

Embryological development is a highly conserved, highly regulated sequence of events in which many processes must be activated or deactivated

in their proper sequence. We are just in the infancy of discovering what are the messages and messengers controlling development of the embryo into a fetus and eventual birth. Many substances perturb these processes and thus are fetotoxic teratogens. The most common human teratogen is alcohol, the use of which during pregnancy can cause the developmental retardation known as the *fetal alcohol syndrome* [33]. Among the many other effects it causes, angiotensin II is a growth regulator. Disturbing angiotensin II action by angiotensin-converting enzyme inhibition, or by angiotensin receptor block can be used therapeutically to reduce the inappropriate growth and remodeling that occurs in congestive heart failure and hypertension. However, these blocking actions also can result in severe fetotoxicity [34]. Numerous other examples of teratogens are known. Screening for these adverse effects is a necessary part of drug discovery.

1.8.8 Miscellaneous Other Mechanisms of Toxicity

Addition of a miscellaneous category to any list adds completeness. On the other hand, it is difficult to find toxic effects that do not fit into one or more of the previous categories. One example of such might be the necrosis of the mandible that appears to result from the use of bisphosphonates to prevent osteoporosis in postmenopausal women. The mechanism of this unexpected effect is not known, but the toxicity certainly has become of great concern to our dental colleagues [35].

1.9 COMPUTATION IN TOXICOLOGY

The primary advantage of the computer is to deal with work that is so large and so complex that it cannot otherwise readily be possible. One example of such a need is the highly complicated chemical/toxicological/biomedical literature that exists. A computer can search and mine the literature, and it can organize it into mutually relevant collections of articles. Data clustering is one example of such an intelligent organization of the literature [36]. Other examples include directory searches, keyword searches, and database searches. (See *types of search engines* [37].)

There are several different clustered search engines available. A case in point is Clusty.com's Vivisimo [38]. The default setting of this engine is to search the recent literature. Repeated searches, say at monthly intervals, enables one to keep up with topics of interest. A recent (June 2006) clustered search on "Computational Toxicology," the topic of this book, gave the results described in Table 1.1.

The relevance of these topics to computation and to toxicology is not trivial. Currently commercially available *gene microarrays* can characterize the expression of thousands of genes of several different species [39], and this

TABLE 1.1 Results of a Clustered Search on “Computational Toxicology” as Divided by the Vivisimo Search Engine into Topic Clusters

Microarray, gene expression profiling (13 articles)
Toxicogenomics (12 articles)
Protein, impact (13 articles)
Dose–response (11 articles)
Quantitative (8 articles)
Receptor, expression (7 articles)
Predict toxicity (7 articles)
Pharmacology and toxicology (6 articles)
Physiologically based pharmacokinetic (8 articles)
Properties (5 articles)
High-throughput data (3 articles)

Note: The search retrieved 105 articles. Less frequent items are not included above.

information has great potential in the drug discovery process [40]. Routines to interpret and correlate these findings are under active development, and the results are available on the internet through the NIH [41]. In the case of *toxicogenomics*, a subset of pharmacogenomics, several correlations between an individual’s genome and susceptibility to particular toxicants are known. Databases exist and people are working to develop toxicogenomic in vitro procedures that might be useful early in drug discovery, or in predictive toxicology [42,43,44].

The *predictive toxicology* cluster provided a group of articles related to QSARs [45], bioinformatics [46], and expert systems [47]. Pharmacokinetic data acquisition and interpretation have been heavily intertwined with computation since the early days of the discipline. This hasn’t changed with the more current field of *toxicokinetics*.

The *high-throughput data* cluster appears to be related to using computational and statistical techniques to separate useful data signals from large amounts of irrelevant noise (e.g., see [48]). This important endeavor is just in its infancy.

The connection between toxicology and *dose–response* relationships is several hundreds of years old [2,49]. In the pre-computational days these data were calculated by hand and nomogram [50]. The sheer labor involved has been greatly eased by computational techniques. Nevertheless, this author feels that working through such manual techniques at least once is very salutary for nascent pharmacologists and toxicologists.

Predictions of drug *receptor* interactions and related QSARs are useful for *predictive toxicology* and drug development. *Validation* of computer technology and predictions is another concern. Many of these topics are covered in the following chapters of this book.

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