CHAPTER 1

Introduction to Toxicology

ERNEST HODGSON

1.1 DEFINITION AND SCOPE, RELATIONSHIP TO OTHER SCIENCES, AND HISTORY

1.1.1 Definition and Scope

Toxicology can be defined as that branch of science that deals with poisons, and a poison can be defined as any substance that causes a harmful effect when administered, either by accident or design, to a living organism. By convention, toxicology also includes the study of harmful effects caused by physical phenomena, such as radiation of various kinds and noise. In practice, however, many complications exist beyond these simple definitions, both in bringing more precise meaning to what constitutes a poison and to the measurement of toxic effects. Broader definitions of toxicology, such as "the study of the detection, occurrence, properties, effects, and regulation of toxic substances," although more descriptive, do not resolve the difficulties. Toxicity itself can rarely, if ever, be defined as a single molecular event but is, rather, a cascade of events starting with exposure, proceeding through distribution and metabolism, and ending with interaction with cellular macromolecules (usually DNA or protein) and the expression of a toxic end point. This sequence may be mitigated by excretion and repair. It is to the complications, and to the science behind them and their resolution, that this textbook is dedicated, particularly to the *how* and *why* certain substances cause disruptions in biologic systems that result in toxic effects. Taken together, these difficulties and their resolution circumscribe the perimeter of the science of toxicology.

The study of toxicology serves society in many ways, not only to protect humans and the environment from the deleterious effects of toxicants but also to facilitate the development of more selective toxicants such as anticancer and other clinical drugs and pesticides.

Poison is a quantitative concept, almost any substance being harmful at some doses but, at the same time, being without harmful effect at some lower dose. Between these two limits there is a range of possible effects, from subtle long-term chronic toxicity to immediate lethality. Vinyl chloride may be taken as an example. It is a potent hepatotoxicant at high doses, a carcinogen with a long latent period at lower

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doses, and apparently without effect at very low doses. Clinical drugs are even more poignant examples because, although therapeutic and highly beneficial at some doses, they are not without deleterious side effects and may be lethal at higher doses. Aspirin (acetylsalicylic acid), for example, is a relatively safe drug at recommended doses and is taken by millions of people worldwide. At the same time, chronic use can cause deleterious effects on the gastric mucosa, and it is fatal at a dose of about 0.2 to 0.5 g/kg. Approximately 15% of reported accidental deaths from poisoning in children result from ingestion of salicylates, particularly aspirin.

The importance of dose is well illustrated by metals that are essential in the diet but are toxic at higher doses. Thus iron, copper, magnesium, cobalt, manganese, and zinc can be present in the diet at too low a level (deficiency), at an appropriate level (maintenance), or at too high a level (toxic). The question of dose-response relationships is fundamental to toxicology (see Section 1.2).

The definition of a poison, or toxicant, also involves a qualitative biological aspect because a compound, toxic to one species or genetic strain, may be relatively harmless to another. For example, carbon tetrachloride, a potent hepatotoxicant in many species, is relatively harmless to the chicken. Certain strains of rabbit can eat *Belladonna* with impunity while others cannot. Compounds may be toxic under some circumstances but not others or, perhaps, toxic in combination with another compound but nontoxic alone. The methylenedioxyphenyl insecticide synergists, such as piperonyl butoxide, are of low toxicity to both insects and mammals when administered alone but are, by virtue of their ability to inhibit xenobiotic-metabolizing enzymes, capable of causing dramatic increases in the toxicity of other compounds.

The measurement of toxicity is also complex. Toxicity may be acute or chronic, and may vary from one organ to another as well as with age, genetics, gender, diet, physiological condition, or the health status of the organism. As opposed to experimental animals, which are highly inbred, genetic variation is a most important factor in human toxicity since the human population is highly outbred and shows extensive genetic variation. Even the simplest measure of toxicity, the LD50 (the dose required to kill 50% of a population under stated conditions) is highly dependent on the extent to which the above variables are controlled. LD50 values, as a result, vary markedly from one laboratory to another.

Exposure of humans and other organisms to toxicants may result from many activities: intentional ingestion, occupational exposure, environmental exposure, as well as accidental and intentional (suicidal or homicidal) poisoning. The toxicity of a particular compound may vary with the portal of entry into the body, whether through the alimentary canal, the lungs, or the skin. Experimental methods of administration such as injection may also give highly variable results; thus the toxicity from intravenous (IV), intraperitoneal (IP), intramuscular (IM), or subcutaneous (SC) injection of a given compound may be quite different. Toxicity may vary as much as tenfold with the route of administration. Following exposure there are multiple possible routes of metabolism, both detoxifying and activating, and multiple possible toxic endpoints (Figure 1.1).

Attempts to define the scope of toxicology, including that which follows, must take into account that the various subdisciplines are not mutually exclusive and are frequently interdependent. Due to overlapping of mechanisms as well as use and chemical classes of toxicants, clear division into subjects of equal extent or importance is not possible.

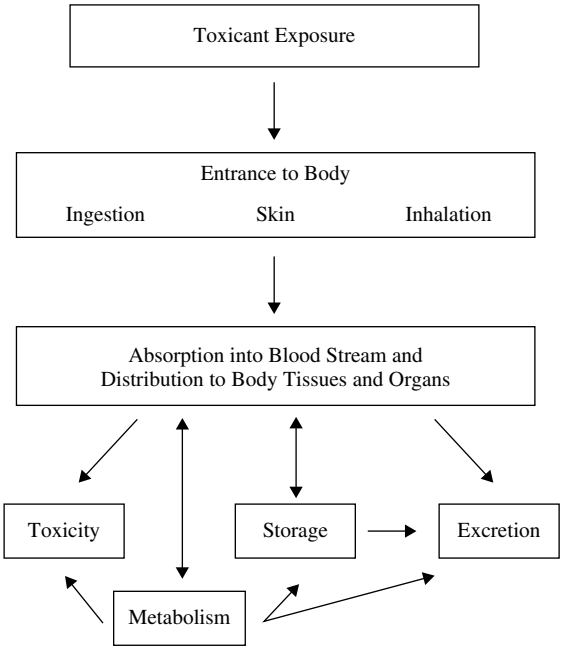

Figure 1.1 Fate and effect of toxicants in the body.

Many specialized terms are used in the various subdisciplines of toxicology as illustrated in the *Dictionary of Toxicology*, 2nd edition (Hodgson et al., 1998). However, some terms are of particular importance to toxicology in general; they are defined in the glossary to be found at the end of this volume.

A. Modes of Toxic Action. This includes the consideration, at the fundamental level of organ, cell and molecular function, of all events leading to toxicity in vivo: uptake, distribution, metabolism, mode of action, and excretion. The term mechanism of toxic action is now more generally used to describe an important molecular event in the cascade of events leading from exposure to toxicity, such as the inhibition of acetylcholinesterase in the toxicity of organophosphorus and carbamate insecticides. Important aspects include the following:

1. *Biochemical and molecular toxicology* consider events at the biochemical and molecular levels, including enzymes that metabolize xenobiotics, generation of reactive intermediates, interaction of xenobiotics or their metabolites with macromolecules, gene expression in metabolism and modes of action, and signaling pathways in toxic action.
2. *Behavioral toxicology* deals with the effects of toxicants on animal and human behavior, which is the final integrated expression of nervous function in the intact animal. This involves both the peripheral and central nervous systems, as well as effects mediated by other organ systems, such as the endocrine glands.
3. *Nutritional toxicology* deals with the effects of diet on the expression of toxicity and with the mechanisms of these effects.

4. *Carcinogenesis* includes the chemical, biochemical, and molecular events that lead to the large number of effects on cell growth collectively known as cancer.
5. *Teratogenesis* includes the chemical, biochemical, and molecular events that lead to deleterious effects on development.
6. *Mutagenesis* is concerned with toxic effects on the genetic material and the inheritance of these effects.
7. *Organ toxicity* considers effects at the level of organ function (neurotoxicity, hepatotoxicity, nephrotoxicity, etc.).

B. Measurement of Toxicants and Toxicity. These important aspects deal primarily with analytical chemistry, bioassay, and applied mathematics; they are designed to provide the methodology to answer certain critically important questions. Is the substance likely to be toxic? What is its chemical identify? How much of it is present? How can we assay its toxic effect, and what is the minimum level at which this toxic effect can be detected? A number of important fields are included:

1. *Analytical toxicology* is a branch of analytical chemistry concerned with the identification and assay of toxic chemicals and their metabolites in biological and environmental materials.
2. *Toxicity testing* involves the use of living systems to estimate toxic effects. It covers the gamut from short-term tests for genotoxicity such as the Ames test and cell culture techniques to the use of intact animals for a variety of tests from acute toxicity to lifetime chronic toxicity. Although the term "bioassay" is used properly only to describe the use of a living organism to quantitate the amount of a particular toxicant present, it is frequently used to describe any in vivo toxicity test.
3. *Toxicologic pathology* is the branch of pathology that deals with the effects of toxic agents manifested as changes in subcellular, cellular, tissue, or organ morphology.
4. *Structure-activity* studies are concerned with the relationship between the chemical and physical properties of a chemical and toxicity and, particularly, the use of such relationships as predictors of toxicity.
5. *Biomathematics and statistics* relate to many areas of toxicology. They deal with data analysis, the determination of significance, and the formulation of risk estimates and predictive models.
6. *Epidemiology* as it applies to toxicology, is of great importance as it deals with the relationship between chemical exposure and human disease in actual populations rather than in experimental settings.

C. Applied Toxicology. This includes the various aspects of toxicology as they apply in the field or the development of new methodology or new selective toxicants for early application in the field setting.

1. *Clinical toxicology* is the diagnosis and treatment of human poisoning.
2. *Veterinary toxicology* is the diagnosis and treatment of poisoning in animals other than humans, particularly livestock and companion animals, but not excluding feral species. Other important concerns of veterinary toxicology are the possible

transmission of toxins to the human population in meat, fish, milk, and other foodstuffs and the care and ethical treatment of experimental animals.

3. *Forensic toxicology* concerns the medicolegal aspects, including detection of poisons in clinical and other samples.
4. *Environmental toxicology* is concerned with the movement of toxicants and their metabolites and degradation products in the environment and in food chains and with the effect of such contaminants on individuals and, especially, populations. Because of the large number of industrial chemicals and possibilities for exposure, as well as the mosaic of overlapping laws that govern such exposure, this area of applied toxicology is well developed.
5. *Industrial toxicology* is a specific area of environmental toxicology that deals with the work environment and constitutes a significant part of *industrial hygiene*.

D. Chemical Use Classes. This includes the toxicology aspects of the development of new chemicals for commercial use. In some of these use classes, toxicity, at least to some organisms, is a desirable trait; in others, it is an undesirable side effect. Use classes are not composed entirely of synthetic chemicals; many natural products are isolated and used for commercial and other purposes and must be subjected to the same toxicity testing as that required for synthetic chemicals. Examples of such natural products include the insecticide, pyrethrin, the clinical drug, digitalis, and the drug of abuse, cocaine.

1. *Agricultural chemicals* include many compounds, such as insecticides, herbicides, fungicides, and rodenticides, in which toxicity to the target organism is a desired quality whereas toxicity to "nontarget species" is to be avoided. Development of such selectively toxic chemicals is one of the applied roles of comparative toxicology.
2. *Clinical drugs* are properly the province of pharmaceutical chemistry and pharmacology. However, toxic side effects and testing for them clearly fall within the science of toxicology.
3. *Drugs of abuse* are chemicals taken for psychological or other effects and may cause dependence and toxicity. Many of these are illegal, but some are of clinical significance when used correctly.
4. *Food additives* are of concern to toxicologists only when they are toxic or being tested for possible toxicity.
5. *Industrial chemicals* are so numerous that testing them for toxicity or controlling exposure to those known to be toxic is a large area of toxicological activity.
6. *Naturally occurring substances* include many phytotoxins, mycotoxins, and minerals, all occurring in the environment. The recently expanded and now extensive use of herbal remedies and dietary supplements has become a cause of concern for toxicologists and regulators. Not only is their efficacy frequently dubious, but their potential toxicity is largely unknown.
7. *Combustion products* are not properly a use class but are a large and important class of toxicants, generated primarily from fuels and other industrial chemicals.

E. Regulatory Toxicology These aspects, concerned with the formulation of laws, and regulations authorized by laws, are intended to minimize the effect of toxic chemicals on human health and the environment.

1. *Legal aspects* are the formulation of laws and regulations and their enforcement. In the United States, enforcement falls under such government agencies as the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA). Similar government agencies exist in many other countries.
2. *Risk assessment* is the definition of risks, potential risks, and the risk-benefit equations necessary for the regulation of toxic substances. Risk assessment is logically followed by *risk communication* and *risk management*.

1.1.2 Relationship to Other Sciences

Toxicology is highly eclectic science and human activity drawing from, and contributing to, a broad spectrum of other sciences and human activities. At one end of the spectrum are those sciences that contribute their methods and philosophical concepts to serve the needs of toxicologists, either in research or in the application of toxicology to human affairs. At the other end of the spectrum are those sciences to which toxicology contributes.

In the first group chemistry, biochemistry, pathology, physiology, epidemiology, immunology, ecology, and biomathematics have long been important while molecular biology has, in the last two or three decades, contributed to dramatic advances in toxicology.

In the group of sciences to which toxicology contributes significantly are such aspects of medicine as forensic medicine, clinical toxicology, pharmacy and pharmacology, public health, and industrial hygiene. Toxicology also contributes in an important way to veterinary medicine, and to such aspects of agriculture as the development and safe use of agricultural chemicals. The contributions of toxicology to environmental studies has become increasingly important in recent years.

Clearly, toxicology is preeminently an applied science, dedicated to the enhancement of the quality of life and the protection of the environment. It is also much more. Frequently the perturbation of normal life processes by toxic chemicals enables us to learn more about the life processes themselves. The use of dinitrophenol and other uncoupling agents to study oxidative phosphorylation and the use of α-amanitin to study RNA polymerases are but two of many examples. The field of toxicology has expanded enormously in recent decades, both in numbers of toxicologists and in accumulated knowledge. This expansion has brought a change from a primarily descriptive science to one which utilizes an extensive range of methodology to study the mechanisms involved in toxic events.

1.1.3 A Brief History of Toxicology

Much of the early history of toxicology has been lost and in much that has survived toxicology is of almost incidental importance in manuscripts dealing primarily with medicine. Some, however, deal more specifically with toxic action or with the use of poisons for judicial execution, suicide or political assassination. Regardless of the paucity of the early record, and given the need for people to avoid toxic animals and plants, toxicology must rank as one of the oldest practical sciences.

The Egyptian papyrus, *Ebers*, dating from about 1500 BC, must rank as the earliest surviving pharmacopeia, and the surviving medical works of Hippocrates, Aristotle,

and Theophrastus published during the period 400 to 250 BC all include some mention of poisons. The early Greek poet Nicander treats, in two poetic works, animal toxins (*Therica*) and antidotes to plant and animal toxins (*Alexipharmica*). The earliest surviving attempt to classify plants according to their toxic and therapeutic effects is that of Dioscorides, a Greek employed by the Roman emperor Nero about AD 50.

There appear to have been few advances in either medicine or toxicology between the time of Galen (AD 131–200) and Paracelsus (1493–1541). It was the latter who, despite frequent confusion between fact and mysticism, laid the groundwork for the later development of modern toxicology by recognizing the importance of the dose-response relationship. His famous statement—"All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and a remedy"—succinctly summarizes that concept. His belief in the value of experimentation was also a break with earlier tradition.

There were some important developments during the eighteenth century. Probably the best known is the publication of Ramazini's *Diseases of Workers* in 1700, which led to his recognition as the father of occupational medicine. The correlation between the occupation of chimney sweeps and scrotal cancer by Percival Pott in 1775 is almost as well known, although it was foreshadowed by Hill's correlation of nasal cancer and snuff use in 1761.

Orfila, a Spaniard working at the University of Paris in the early nineteenth century, is generally regarded as the father of modern toxicology. He clearly identified toxicology as a separate science and, in 1815, published the first book devoted exclusively to toxicology. An English translation in 1817, was entitled *A General System of Toxicology or, A Treatise on Poisons, Found in the Mineral, Vegetable and Animal Kingdoms, Considered in Their Relations with Physiology, Pathology and Medical Jurisprudence*. Workers of the late nineteenth century who produced treatises on toxicology include Christian, Kobert, and Lewin. The recognition of the site of action of curare by Claude Bernard (1813–1878) began the modern study of the mechanisms of toxic action. Since then, advances have been numerous—too numerous to list in detail. They have increased our knowledge of the chemistry of poisons, the treatment of poisoning, the analysis of toxicants and toxicity, modes of toxic action and detoxication processes, as well as specific molecular events in the poisoning process.

With the publication of her controversial book, *The Silent Spring*, in 1962, Rachel Carson became an important influence in initiating the modern era of environmental toxicology. Her book emphasized stopping the widespread, indiscriminate use of pesticides and other chemicals and advocated use patterns based on sound ecology. Although sometimes inaccurate and with arguments often based on frankly anecdotal evidence, her book is often credited as the catalyst leading to the establishment of the US Environmental Protection Agency and she is regarded, by many, as the mother of the environmental movement.

It is clear, however, that since the 1960s toxicology has entered a phase of rapid development and has changed from a science that was largely descriptive to one in which the importance of mechanisms of toxic action is generally recognized. Since the 1970s, with increased emphasis on the use of the techniques of molecular biology, the pace of change has increased even further, and significant advances have been made in many areas, including chemical carcinogenesis and xenobiotic metabolism, among many others.

1.2 DOSE-RESPONSE RELATIONSHIPS

As mentioned previously, toxicity is a relative event that depends not only on the toxic properties of the chemical and the dose administered but also on individual and interspecific variation in the metabolic processing of the chemical. The first recognition of the relationship between the dose of a compound and the response elicited has been attributed to Paracelsus (see Section 1.1.3). It is noteworthy that his statement includes not only that all substances can be toxic at some dose but that "the right dose differentiates a poison from a remedy," a concept that is the basis for pharmaceutical therapy.

A typical dose-response curve is shown in Figure 1.2, in which the percentage of organisms or systems responding to a chemical is plotted against the dose. For many chemicals and effects there will be a dose below which no effect or response is observed. This is known as the *threshold dose*. This concept is of significance because it implies that a *no observed effect level* (NOEL) can be determined and that this value can be used to determine the safe intake for food additives and contaminants such as pesticides. Although this is generally accepted for most types of chemicals and toxic effects, for chemical carcinogens acting by a genotoxic mechanism the shape of the curve is controversial and for regulatory purposes their effect is assumed to be a no-threshold phenomenon. Dose-response relationships are discussed in more detail in Chapter 21 on toxicity testing.

1.3 SOURCES OF TOXIC COMPOUNDS

Given the enormous number of toxicants, it is difficult to classify them chemically, either by function or by mode of action, since many of them would fall into several classes. Some are natural products, many are synthetic organic chemicals of use to society, while others are by-products of industrial processes and waste disposal. It is useful, however, to categorize them according to the expected routes of exposure or according to their uses.

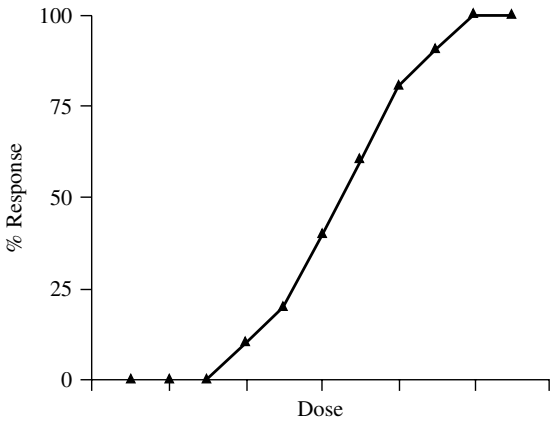

Figure 1.2 A typical dose-response curve.

1.3.1 Exposure Classes

Exposure classes include toxicants in food, air, water, and soil as well as toxicants characteristic of domestic and occupational Settings. Toxicant exposure classes are described in detail in Chapter 4.

1.3.2 Use Classes

Use classes include drugs of abuse, therapeutic drugs, agricultural chemicals, food additives and contaminants, metals, solvents, combustion products, cosmetics, and toxins. Some of these, such as combustion products, are the products of use processes rather than being use classes. All of these groups of chemicals are discussed in detail in Chapter 5.

1.4 MOVEMENT OF TOXICANTS IN THE ENVIRONMENT

Chemicals released into the environment rarely remain in the form, or at the location, of release. For example, agricultural chemicals used as sprays may drift from the point of application as air contaminants or enter runoff water as water contaminants. Many of these chemicals are susceptible to fungal or bacterial degradation and are rapidly detoxified, frequently being broken down to products that can enter the carbon, nitrogen, and oxygen cycles. Other agricultural chemicals, particularly halogenated organic compounds, are recalcitrant to a greater or lesser degree to metabolism by microorganisms and persist in soil and water as contaminants; they may enter biologic food chains and move to higher trophic levels or persist in processed crops as food contaminants. This same scenario is applicable to any toxicant released into the environment for a specific use or as a result of industrial processes, combustion, and so on. Chemicals released into the environment are also susceptible to chemical degradation, a process often stimulated by ultraviolet light.

Although most transport between inanimate phases of the environment results in wider dissemination, at the same time dilution of the toxicant in question and transfer among living creatures may result in increased concentration or bioaccumulation. Lipid soluble toxicants are readily taken up by organisms following exposure in air, water, or soil. Unless rapidly metabolized, they persist in the tissues long enough to be transferred to the next trophic level. At each level the lipophilic toxicant tends to be retained while the bulk of the food is digested, utilized, and excreted, thus increasing the toxicant concentration. At some point in the chain, the toxicant can become deleterious, particularly if the organism at that level is more susceptible than those at the level preceding it. Thus the eggshell thinning in certain raptorial birds was almost certainly due to the uptake of DDT and DDE and their particular susceptibility to this type of toxicity. Simplified food chains are shown in Figure 1.3.

It is clear that such transport can occur through both aquatic and terrestrial food chains, although in the former, higher members of the chains, such as fish, can accumulate large amounts of toxicants directly from the medium. This accumulation occurs because of the large area of gill filaments, their intimate contact with the water and the high flow rate of water over them. Given these characteristics and a toxicant with a high partition coefficient between lipid membranes and water, considerable uptake is inevitable.

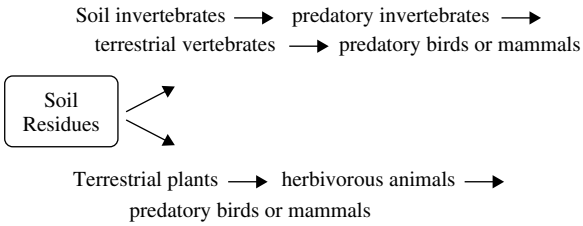

Figure 1.3 Examples of simplified food chains.

These and all other environmental aspects of toxicology are discussed in Part VII.

SUGGESTED READING

Hodgson, E., and R. C. Smart, eds. *Introduction to Biochemical Toxicology*, 3rd ed. New York: Wiley, 2001.

Hodgson, E., R. B. Mailman, and J. E. Chambers, eds. *Dictionary of Toxicology*, 2nd ed. London: Macmillan, 1998.

Klaassen, C. D. ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 6th ed. New York: McGraw-Hill, 2001.

Timbrell, J. A. *Principles of Biochemical Toxicology*, 3rd ed. London: Taylor and Francis, 2000.

Wexler, P. *Information Resources in Toxicology*, 3rd ed. San Diego: Academic Press, 2000.

CHAPTER 2

Introduction to Biochemical and Molecular Methods in Toxicology

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2.1 INTRODUCTION

This chapter is not designed to summarize biochemical methods long used in toxicology such as colorimetric and radiometric methods for the investigation of xenobiotic metabolism, either in vivo or in vitro, but rather to give a brief summary of the methods of molecular and cellular biology that have become, more recently, of critical importance in toxicological research. The chapter owes much to Chapters 2 through 4 of the third edition of *Introduction to Biochemical Toxicology* (see Suggested Reading), and the reader is referred to these chapters for additional information.

2.2 CELL CULTURE TECHNIQUES

While scientists have had the ability to culture many unicellular organisms for some time, recent advances in the culture of cells from multicellular organisms have played a pivotal role in recent advances in toxicology. Cells can be isolated and either maintained in a viable state for enough time to conduct informative experiments or, in some cases, propagated in culture. The advantages of cultured cells are that they can provide living systems for the investigation of toxicity that are simplified relative to the intact organism and they can be used as replacements for whole animal toxicity testing if the toxic end point can be validated. Human cells play an important role in the extrapolation of toxic effects, discovered in experimental animals, to humans. Cultured cells, from humans or other mammals, are utilized in many of the molecular methods mentioned below. There are, however, limitations in the use of cellular methods. It has not been possible to culture many cell types, and of those that have been cultured, the loss of differentiated cell function is a common problem. Extrapolation of findings to the intact animal is often problematical and the use of undefined media constituents such as serum, often essential for cell viability, may have unwanted or undefined effects on cell function and toxicant bioavailability.

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Studies have been carried out on cells isolated from tissues and maintained in suspension culture or on cells that have formed monolayers.

2.2.1 Suspension Cell Culture

Circulating blood cells or cells easily obtained by lavage such as peritoneal and alveolar macrophages can normally survive in suspension culture when provided with a suitable nutrient medium. Cells from organized solid organs or tissues must be separated from the tissue and, if possible, separated into cell types, before being suspended in such a medium.

Cell association within organs depends on protein complex formation, which in turn is Ca^{2+} dependent. Consequently dissociation media generally contain a proteolytic enzyme and the Ca^{2+} chelator EDTA. There are a number of methods available to separate cell types from the mixture of dispersed cells, the commonest being centrifugation without a density gradient, wherein cells are separated by size, or centrifugation through a density gradient wherein cells are separated on the basis of their buoyant density.

Cells in suspension may be maintained for a limited period of time in defined media or for longer periods in nutrient, but less well-defined, media. In either case these cultures are often used for studies of xenobiotic metabolism.

2.2.2 Monolayer Cell Culture

Proliferation of most cells in culture requires attachment to a substrate and occurs until limited by cell-to-cell contact, resulting in the formation of a cellular monolayer. The substrate provided for attachment is usually polystyrene modified to carry a charge. The medium for continued maintenance and growth contains salts and glucose, usually with a bicarbonate buffer. Because of the bicarbonate buffering system these cultures are maintained in a 5–10% CO_2 atmosphere in a temperature and humidity controlled incubator. Many cells require serum for optimal growth, inducing considerable variability into the experimental system. Since the factors provided by serum are numerous and complex, defined serum substitutes are not always successful. The factors provided by serum include proteins such as growth factors, insulin and transferrin (to provide available iron), small organic molecules such as ethanolamine, and pyruvate and inorganic ions, such as selenium.

2.2.3 Indicators of Toxicity in Cultured Cells

Routine observation of cultured is usually carried out by phase contrast microscopy, utilizing the inverted phase contrast microscope. More recently, more detailed observations have become possible utilizing fluorescent tags and inverted fluorescent microscopes. Fluorescent tags currently in use permit the assessment of oxidant status and mitochondrial function as well as the intracellular concentration of sulfhydryl groups, Ca^{2+}, H^+, Na^+, and K^+.

Toxicity to cultured cells may be the result either of inadequacies in the culture or the toxicity effects of the chemical being investigated. Short-term toxicity is usually

Table 2.1 Examples of Application of Cell Lines Retaining Differentiated Properties in the Study of Toxic Effects*

Cell Line	Source	Differentiated Cell Type	Toxicant	Measured End Point
N1E-115	Mouse neuroblastoma	Cholinergic neuron	Lead	Blockage of voltage-dependent Ca^{2+} channels
			Pyrethroid insecticide	Prolonged open time for voltage-dependent Na^{+} channels
PC12	Rat pheochromocytoma (adrenal medullary tumor)	Adrenergic neuron	Tricresyl phosphate (organophosphate)	Inhibition of neurofilament assembly and axonal growth
SK-N-S11	Human neuroblastoma	Neuron	N_2O (anesthetic)	Depressed cholinergic Ca^{2+} signaling
Hepa-1	Mouse hepatoma	Hepatocyte	2,3,7,8-terachloro-dibenzodioxane (TCCD)	Induction of CYP1A1 and 1B1
H114 E	Rat hepatoma	Hepatocyte	Polychlorinated biphenyls (PCBs)	Induction of CYP1A1
HepG2	Human hepatoblastoma	Hepatocyte	Cyclophosphamide (antineoplastic)	Cytochrome P450-dependent genotoxicity
3T3-L1	Mouse embryo fibroblasts	Adipocytes	TCDD	Inhibition of glucose transport and lipoprotein lipase
Y1	Mouse adrenocortical tumor	Adrenocortical cell	Methyl sulfone metabolites of DDT and PCBs	Inhibition of corticosterone synthesis by competitive inhibition of cytochrome P450
LLC-PK1	Pig kidney	Renal tubule epithelial cell	Cadmium	Cytotoxicity, apoptosis
MDCK	Dog kidney	Renal tubule epithelial cell	Organic mercury compounds	Cytotoxicity, transepithelial leakiness

Source: E. Hodgson and R. C. Smart, eds., *An Introduction to Modern Toxicology*, 3rd ed. New York: Wiley, 2001.

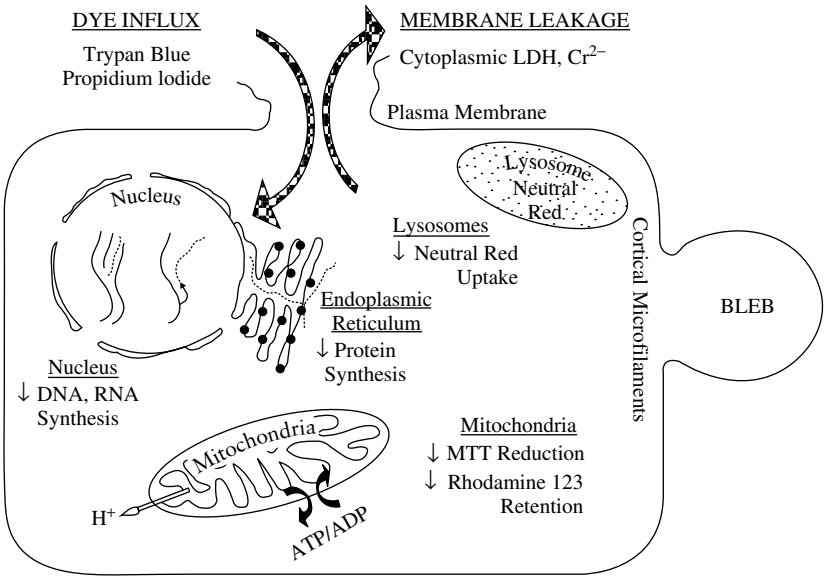

Figure 2.1 Idealized diagram of a cell to illustrate parameters often used to measure cytotoxicity and the corresponding affected subcellular organelle. (From *An Introduction to Biochemical Toxicology*, 3rd ed., E. Hodgson and R. C. Smart, eds., Wiley, 2001.)

evaluated by examination of end points that indicate effects on cellular organelles such as leakage of cell constituents into the medium, uptake of dyes into the cell and the formation of surface "blebs." This is illustrated in Figure 2.1.

Longer term assessments of cell toxicity are highly dependent on the relevant toxic end point. They may include measurement of growth competence, apoptosis, and/or necrosis, incorporation of radioactive precursors into essential cellular constituents such as RNA, DNA, and protein and specialized cellular functions. Some examples of the use of cultured cell lines in the study of toxicity effects are shown in Table 2.1.

2.3 MOLECULAR TECHNIQUES

Recombinant DNA techniques, including molecular cloning, have provided recent dramatic advances in many areas of both fundamental and applied biology, toxicology not excepted. Responses to toxicants may involve changes in gene expression and the new microarray techniques enable the simultaneous examination of the level of expression of many genes. The completion of the Human Genome Project will permit toxic effects in humans to be investigated and will facilitate extrapolation from experimental animals. The human genome will also provide the essential genetic background information for studies of polymorphisms in xenobiotic-metabolizing and other enzymes. Such polymorphisms have already been shown to be very important in individual sensitivity to clinical drugs and in the definition of populations and/or individuals at increased risk from particular toxicants. Chemically induced mutations, particularly in oncogenes and tumor-suppressor genes are important in chemical carcinogenesis. The

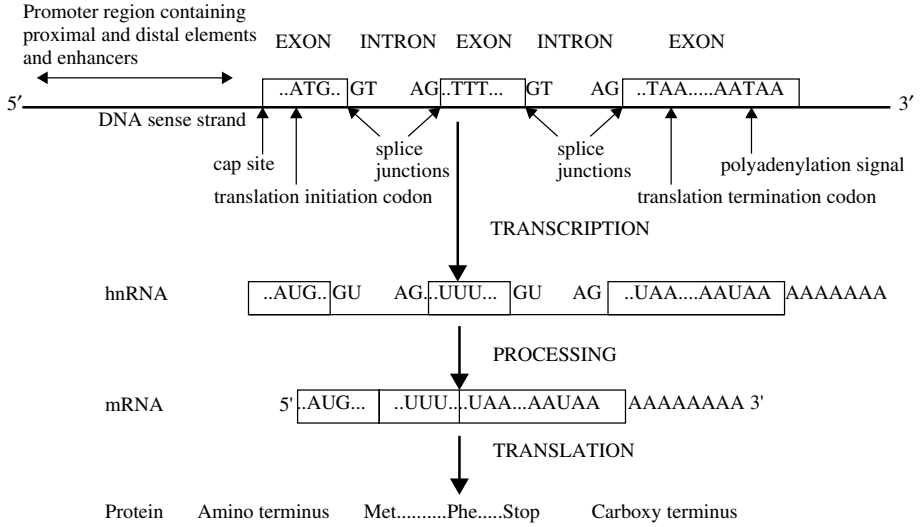

Figure 2.2 Transcription, mRNA processing, and translation. DNA sense strand is designated by bold lines, hnRNA and mRNA by thinner lines. Exons are shown as rectangles and introns as the intervening spaces between exons. (From *An Introduction to Biochemical Toxicology*, 3rd edition, E. Hodgson and R. C. Smart, eds., Wiley, 2001.)

ability to develop "knockout" animals lacking a particular gene and transgenic animals with an additional transgene is also proving important in toxicological studies.

Gene structure and any of the processes involved in DNA expression including transcription, mRNA processing and translation and protein synthesis (Figure 2.2) can all be examined by molecular techniques. In toxicology this may include toxic effects on these processes or the role of the processes in the mechanism of toxic action.

2.3.1 Molecular Cloning

The basic principle of molecular cloning is the insertion of a DNA segment into a suitable vector. The vector is an autonomously replicating DNA molecule and the inserted DNA segment may be as large as a gene or a small as a few nucleotides. The vector containing the DNA is inserted into a cell such as yeast, where it can be replicated many times, and either the DNA or the expressed protein subsequently isolated (Figure 2.3).

2.3.2 cDNA and Genomic Libraries

cDNA or genomic libraries are collections of DNA fragments incorporated into a recombinant vector and transformed into an appropriate host cell. In the case of cDNA libraries, the cDNAs complementary to all of the mRNAs in the tissue or cell sample are synthesized in a procedure using reverse transcriptase, before incorporation into the vector. With genomic DNA libraries the genomic DNA is digested, before cloning into the vector, with a restriction enzyme to produce an overlapping set of DNA fragments of some 12 to 20 kb.

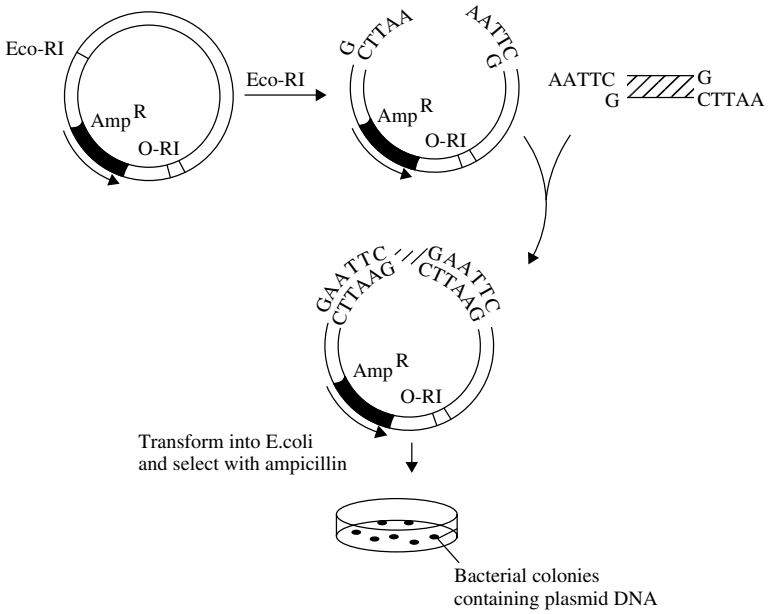

Figure 2.3 Molecular cloning using a plasmid vector. (From *An Introduction to Biochemical Toxicology*, 3rd ed., E. Hodgson and R. C. Smart, eds., Wiley, 2001.)

These libraries are used in many screening procedures and many transgenic proteins now routinely available were obtained by their use. Although in some applications the use of cDNA and genomic libraries has been superceded by other methods, particularly those based on PCR, they are still used to advantage in many applications.

2.3.3 Northern and Southern Blot Analyses

Northern analysis is usually used to identify and quantitate specific mRNAs in a sample. Southern analysis is used to determine whether or not a gene of interest is present as well as its copy number. Other uses for Southern analysis include identifying restriction fragment length polymorphisms and changes in heterozygosity.

In both Southern and Northern analyses restriction-digested DNA fragments, mRNA, and polyA mRNA are separated by size when electrophoresed on agarose gel. The separated molecules are transferred, by electroblotting or capillary blotting, on to a nylon or nitrocellulose membrane. The immobilized RNA or DNA is reacted with a radiolabeled, chemiluminescent, or fluorescent probe that is complementary to the DNA/RNA of interest, unbound probe is washed off, and the membrane exposed, in the case of radioactive probes, to radioautographic film to visualize the sample of interest.

2.3.4 Polymerase Chain Reaction (PCR)

PCR is a powerful technique that can, starting with amounts of DNA as small as those found in single cells, amplify the DNA until large amounts are available for many

different kinds of research. Twenty to 40 cycles of can provide up to 10^5 times the original DNA sample.

It is necessary to know as much of the sequence of the DNA of interest as possible in order to construct appropriate primers. These primers are complementary to the sequence at each end of the DNA sequence to be amplified. The DNA is incubated in a thermal cycler with thermostable DNA polymerase, all four dNTP, and the primers. The incubation temperature is raised to separate the DNA strands, lowered to permit annealing of the primers to the complementary regions of the DNA and then raised to permit the polymerase to synthesize DNA. This cycle is then repeated up to 40 times. The PCR technique has been used for many types of toxicological investigation including; uncovering polymorphisms in xenobiotic-metabolizing enzymes, isolating genes from cDNA and genomic libraries and for mutational analysis, to name only a few.

2.3.5 Evaluation of Gene Expression, Regulation, and Function

The methods used for the evaluation of regulation of gene expression are too numerous to be described in detail here. They include Northern analysis to determine levels of a particular mRNA, nuclear run on to determine whether an increase in mRNA is due to an increase in the rate of transcription, and promoter deletion analysis to identify specific elements in the promoter region responsible for the control of expression. Of much current interest is the use of microarrays that permit the study of the expression of hundreds to thousands of genes at the same time. Reverse transcriptase–polymerase chain reaction and RNase protection assay techniques are used to amplify and quantitate mRNAs, while the electrophoretic mobility shift assay is used to measure binding of a transcription factor to its specific DNA consensus sequence.

Gene function in cultured cells can be investigated by expression of the gene product in a suitable expression system or, in vivo, by the creation of transgenic mice, either knockout mice in which the gene in question has been functionally deleted or mice into which a transgene has been introduced.

A general, but more detailed and specific, account of these methods may be found in Smart (2001; see Suggested Reading).

2.4 IMMUNOCHEMICAL TECHNIQUES

Most of the recently developed methods for the detection, characterization, and quantitation of proteins are immunoassays based on the fact that proteins are antigens, compounds that can be recognized by an antibody. It is also true that by combining small molecules (haptens) with a larger carrier molecule such as a protein, these methods can be extended to small molecules of interest since antibodies can be produced that recognize epitopes (specific sites on the antigen recognized by the antibody) that include the hapten.

The antibodies used may be polyclonal or monoclonal, each with characteristics fitting them for use in particular immunochemical methods. Injection of a mammal with a foreign protein (immunogen) gives rise to an immune reaction that includes the generation of antibodies from B lymphocytes. Each B lymphocyte gives rise to only a single antibody type that recognizes a single epitope on the antigen. However,

since these antibodies are derived from many different B lymphocytes the mixture of antibodies can recognize and bind to many different epitopes on the antigen. This mixture of antibodies can be isolated from the serum of the treated animal and is known, collectively, as *polyclonal antibodies.* However, if individual B lymphocytes from a treated animal can be isolated and cultured, because they are of a single clonal origin, they will produce a specific *monoclonal antibody* that recognizes only a single epitope on the antigen (Figure 2.4). Because of the multiple sites for binding polyclonal antibodies are highly reactive. They are also relatively easy to produce. Monoclonal antibodies, although more difficult to produce, are, on the other hand, more specific. The advantages and disadvantages of each must be considered to determine which is the antibody of choice for a particular application. The most important immunochemical methods include the following:

Immunolocalization is a technique for identifying the presence of a protein within the cell, its relative abundance and its subcellular localization. After suitable preparation of the cells, they are treated with an antibody (the primary antibody) that binds to the protein of interest. An antibody that binds to the primary antibody (the secondary antibody) is then allowed to bind and form an antigen—primary antibody—secondary antibody complex. The detection system generally consists of the formation of a colored insoluble product of an enzymatic reaction, the enzyme, such as alkaline phosphatase or horseradish peroxidase, being covalently linked to the secondary antibody.

Immunoaffinity purification involves the use of antibodies, bound to an insoluble matrix, for chromatography. The advantage of this method is that it is highly specific, often permitting purification in a single step. *Immunoprecipitation* is a variant of immunoaffinity purification and is a means to remove a protein from a complex mixture in a highly specific manner.

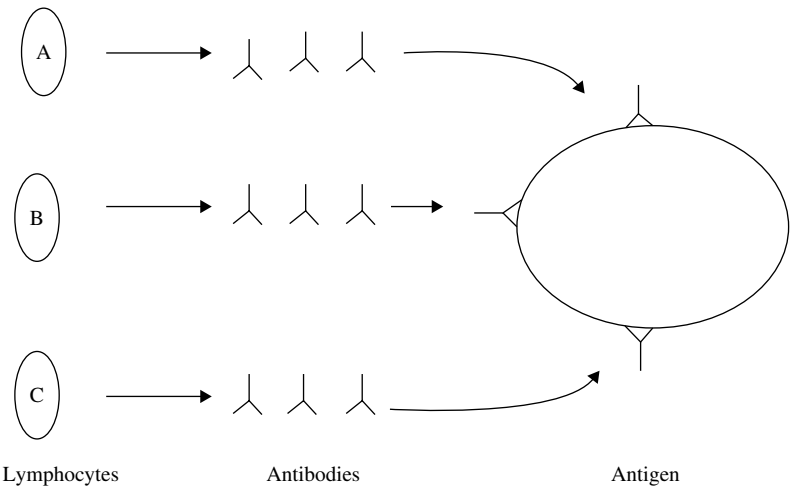

Figure 2.4 The generation of antibodies of several clonal origins (polyclonal antibodies) with antibodies from each clonal origin (monoclonal antibodies A, B and C) recognizing a distinct epitope on the antigen. (From *An Introduction to Biochemical Toxicology*, 3rd ed., E. Hodgson and R. C. Smart, eds., Wiley, 2001.)

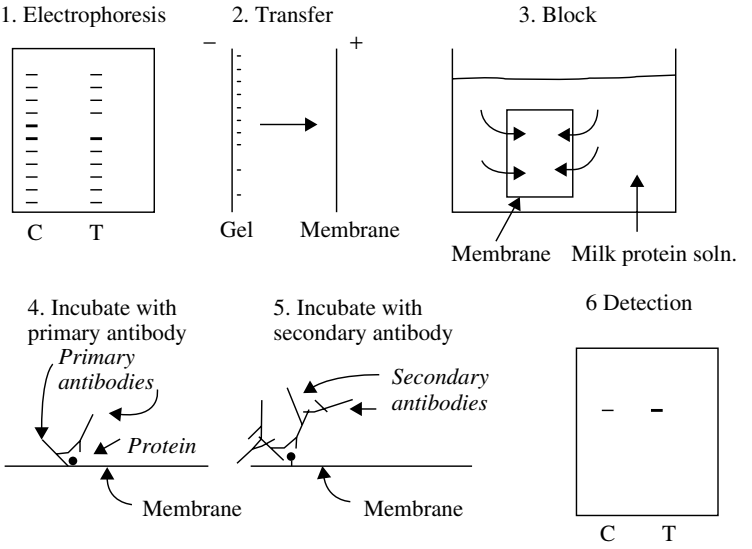

Figure 2.5 Diagrammatic representation of the use of immunoblotting to assess relative levels of a P450 protein following treatment of rats with a PCB. C = hepatic microsomal proteins from a control, untreated rat; T = hepatic microsomal proteins from a rat treated with PCBs. (From *An Introduction to Biochemical Toxicology*, 3rd ed., E. Hodgson and R. C. Smart, eds., Wiley, 2001.)

Western blotting is a widely used technique in which antibodies are used to detect proteins following electrophoresis, generally SDS polyacrylamide gel electrophoresis that permits the separation of proteins on the basis of their molecular weights (Figure 2.5). Western blotting can be used to determine the presence and relative amount of a particular protein in a biological sample as well as its molecular weight.

Radioimmunoassay (RIA) is a very sensitive method used to measure minute quantities of an antigen. Since this method is most often used to measure drugs, toxicants, and other xenobiotics, the antigen used to produce the antibody is the small molecule (hapten) linked covalently to a protein. Among the techniques used in the actual measurement, the antigen capture method, in which the competition between radiolabeled antigen and the unlabeled antigen in the sample, is the most common.

Depending on the design of the method *enzyme-linked immunoabsorbant assays* (ELISA) can be used to measure either antigens or antibodies in mixtures by using enzymatic-mediated detection of the corresponding immobilized immune complex. Even though this method has proved to be most useful for the rapid estimation of antibodies or antigens in complex biological mixtures, it has also been used for the quantitation of small molecules in a manner analogous to radioimmunoassays.

Inhibitory antibodies are frequently used in studies of xenobiotic metabolism, usually to estimate the contribution of particular enzymes in multienzyme mixtures. An important example is the use of antibodies to estimate the contribution of individual cytochrome P450 isoforms to the overall metabolism of a xenobiotic in microsomal preparations.

SUGGESTED READING

Hodgson, E., and R. C. Smart, eds. *Introduction to Biochemical Toxicology*, 3rd ed. New York: Wiley, 2001. Relevant chapters include:

Chapter 2. Smart, R. C. Overview of molecular techniques in toxicology: genes/transgenes.

Chapter 3. LeBlanc, G. A. Immunochemical techniques in toxicology.

Chapter 4. Meyer, S. A. Overview of cellular techniques in toxicology.

CHAPTER 3

Toxicant Analysis and Quality Assurance Principles

ROSS B. LEIDY

3.1 INTRODUCTION

Today's analytical chemist is armed with an amazing number of tools for the determination and quantitation of potentially toxic compounds at concentrations thought to be impossible to detect 10 years ago. Those involved in the analyses of samples containing, for example, carcinogens in tissue samples, pesticide residues from soils, and polycyclic aromatic hydrocarbons (PAHs) from air, must have a solid understanding of all procedural aspects related to the samples that they analyze to ensure that the data generated are accurate and complete. For example, the ability to determine residues of toxicants in environmental matrices (e.g., food, soil, and water) is crucial to support efforts that are designed to protect both the environment and human health. Toxicologists who are involved in interpreting or reviewing data from studies conducted in the pharmaceutical or agrochemical industries must be familiar with the associated quality assurance and quality control aspects to ensure that the completed data package contains all of the information required by federal and state regulations. To provide the required data, certain approaches to chemical analysis must be followed. This chapter is designed to provide a brief overview of these approaches, including the importance of each step and how a failure to properly consider and follow accepted practices can result in flawed enforcement or regulatory decisions. A more detailed description of sampling, extraction and cleanup procedures, including the primary analytical instruments used to quantitate concentrations, will be discussed in Chapter 25.

3.2 GENERAL POLICIES RELATED TO ANALYTICAL LABORATORIES

Every analytical laboratory, governmental, private, or university, has a standard set of procedures that provide both general and specific information to laboratory members. These fall into certain categories, including the laboratory's standard operating procedures (SOPs), quality assurance/quality control manuals (QA/QC manuals), procedural manuals, analytical method files, and laboratory information management systems

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(LIMSs). These documents/computer programs are designed to standardize all of the aspects of analyses so that a logical, planned, series of events can be followed. Policies generally are reviewed yearly, and the input provided by the laboratory team enhances the efficiency and proficiency of the members to generate accurate results in a timely manner.

3.2.1 Standard Operating Procedures (SOPs)

Over the years members of the laboratory staff have developed routine ways of doing things such as the proper washing of glassware or how many fortifications are done per unit of samples (e.g., 1/10 samples). This set of documents, gathered into the standard operating procedures (SOPs) manual, provides details for both new and experienced chemists on procedures that are performed routinely by the laboratory members. The key word associated with SOPs is routine, because they deal with laboratory procedures that are ancillary to the actual analyses of samples. A laboratory should have SOPs for sample handling, storage, maintenance, replacement of laboratory chemicals, solvents and standards, and the use of laboratory equipment such as pH meters. However, as equipment such as pH meters are replaced with newer models and new systems (e.g., high-speed blenders) are introduced, set procedures must be developed for each. Thus, any SOP is a "living document" that is routinely updated (usually yearly) by the laboratory director with input by all laboratory members.

3.2.2 QA/QC Manuals

Sets of instructions that detail the procedures designed to reduce errors occurring during analytical procedures and ensure accurate quantitations are found in the quality assurance (QA) and quality control (QC) manuals. Quality assurance procedures are used by the laboratory to detect and correct problems in analytical processes. As newer methods and instrumentation are added to the laboratory, older procedures must be modified or changed completely. Quality control procedures are used to maintain a measurement system (i.e., a gas chromatograph) in a statistically satisfactory state to ensure the production of accurate and reliable data.

3.2.3 Procedural Manuals

Beginning chemists must be taught how to use the equipment that they will work with on a daily basis. Questions such as "How does one set the voltage on an electron capture detector?" will be answered initially by the more experienced chemists. However, later they might not be available, so where does one turn if unfamiliar equipment is being used? Many laboratories have procedural manuals that provide detailed information on using the various pieces of equipment that are found in a modern analytical laboratory. They provide step-by-step instructions on using these items and are a valuable reference guide to their proper use. In addition, placing the manufacturer's procedural manual with the laboratory-generated instruction sheet should provide detailed information on troubleshooting, a parts list, telephone numbers, and Internet sites. Like the other manuals listed above, these must be updated on a routine schedule, and as new equipment is procured to replace existing items, new instructions must be added and old information removed.

3.2.4 Analytical Methods Files

All laboratories maintain files containing analytical methods for sample matrices thought to contain specific toxicants that are analyzed routinely. If the US Environmental Protection Agency (USEPA) or manufacturer's methods are used and have been modified by the laboratory, the modifications can be highlighted followed by a brief discussion as to why the modifications were made. The objective is to provide insight into the logic of why the modification was made. Files can be arranged by sample matrix as a general category, and subdivided by compound type (e.g., organophosphate), then further subdivided by specific compound (e.g., diazinon). If kept up to date, these files provide a large amount of information at all levels of experience.

3.2.5 Laboratory Information Management System (LIMS)

As computers and software became more sophisticated, it was only a matter of time before attention was turned to laboratory management. Through large databases, applications were designed to manage and store information associated with a laboratory, and to include many unique features. These laboratory information management systems (LIMS) have found widespread use in regulatory laboratories. A typical LIMS contains the following functions:

1. A sample tracking module, using a unique computer-generated identification number, that allows sample tracking from the time the sample enters the laboratory until it is analyzed.
2. A sample scheduling module that automatically logs in the sample, prints barcoded labels and assigns analyses for routine projects.
3. A personnel and equipment module that maintains employee training records, tracks instrument calibration, repairs, costs, and so on.
4. A data entry module that allows the chemist to enter results into the LIMS, assign QC runs, and prepare reports.
5. A QA/QC module that allows generation of control charts and produces graphs that detail trends.
6. An electronic data transfer module that automatically transfers data generated from the electronics of the analytical instrument into the LIMS, thus reducing transcription errors.
7. A chemical and reagent inventory module that manages the purchase and use of laboratory supplies, keeps track of lot number purchases, shelf lives, and costs.
8. A maintenance module that allows laboratory managers to manage the database, monitor studies, methods, priorities of projects, and so on.

LIMS have become more sophisticated and recently have been combined with two newly developed systems, the chemical information management system (CIMS), which searches for, manipulates, and stores chemical structures, reactions, and associated data, and the analytical information management system (AIMS), which acts as a central repository for instrumental data (e.g., chromatograms).

3.3 ANALYTICAL MEASUREMENT SYSTEM

Toxicants are generally found at low concentrations (e.g., ppm or ppb) regardless of the sample matrix being evaluated. These concentrations are based on the measurement of a response from some instrument to the compound(s) of interest from an extract of the sample matrix. Thus it is necessary to have a system capable of measuring the compound of interest, and in order to ensure the reliability of the data, the analytical process (instrument and analytical method) must be monitored closely.

This measurement process involves much more than injecting some amount of the extracted sample and comparing its response to that of a standard of known concentration. Analytical standards must be prepared, weighed, and diluted carefully to ensure that the concentrations reported reflect those found in the sample analyzed. In addition the analytical instrument used must be calibrated properly to ensure accuracy. Essentially this involves two processes: calibration of the detector against the compound of interest in order to eliminate or minimize any deviation (bias) of response in one direction or another from that expected from previous experience or expected results. Second, calibration of the total analytical system using statistical approaches to minimize bias in determining the presence or absence of the analyte being sought.

3.3.1 Analytical Instrument Calibration

In setting up instrument parameters, consider what is involved in determining residue levels of an analyte. The data produced are only as good as the extract derived from the original sample. If the analyte is distributed uniformly over the area sampled, the concentrations found will be equal, regardless of where the sample is taken. Along these same lines, the analytical procedure will result in uniform residue values if all procedures and instrument parameters remain the same. Based on experience, we know that this distribution of residue over an area will vary as will the analytical procedures and instrument parameters. If we increase the number of samples collected and analyzed, the differences observed will tend to get smaller, resulting in a mean or average value that locates the center of the distribution. Ideally this distribution is called a normal distribution or Gaussian distribution, and looks like a bell (the classic "bell-shaped curve") when the parameters being measured are plotted on a graph (i.e., frequency vs. concentration). Second, the difference in individual measurement, called the standard deviation (σ), defines the variation found in individual measurements. Equations and tables have been developed to determine the significance of suspected deviations and are used to confirm the presence of a suspected problem. If an infinite number of samples from the area are collected and analyzed, the variation in 95% of the samples will cover the true population percentage.

3.3.2 Quantitation Approaches and Techniques

Quantitation is an extremely important part of the analysis to the residue chemist. Residue analysis involves the removal of the compound of interest from some sample matrix. Accurate results come from being thoroughly familiar with the procedures involved in order to establish and maintain appropriate tolerances. The most fundamental decision made is whether the analyte is present or absent, particularly when its

concentration is at or close to its detection limit. Since the measurements are derivations of a known relationship between the analyte concentration and the magnitude of the signal made by the instrument, there is additional signal (noise) generated from the presence of co-extractives, instrument noise, and the like. The analyst uses this "contaminated" signal to decide whether the analyte is present or absent, and selects one of these choices. The decision process is subject to two types of errors: the analyte is present when actually it is not, and the analyte is absent when actually it is present. The terminology for these decision processes are commonly called "false positives" and "false negatives," respectively.

3.4 QUALITY ASSURANCE (QA) PROCEDURES

Over the last 20 years the reliability of data produced by analytical laboratories has increased dramatically. Strict requirements have ensured that the data were produced under defined standards of quality with a stated level of confidence. The routine day-to-day activities (e.g., matrix fortifications) to control, assess, and ensure the quality of generated data are the quality controls associated with analytical processes. The management of the system that ensures that these processes are in place and functional is the quality assurance portion of the laboratory program to produce reliable data.

Quality assurance (QA) is an essential part of analytical protocols. Each laboratory is required to detect and correct problems in analytical processes and to reduce errors to agreed-upon limits. To produce data that have acceptable quality, all laboratory members must follow established guidelines and protocols. Some of the essential elements that must be included in a QA program are as follows:

1. Laboratory practices (e.g., glass washing protocols) must be developed, reviewed, and updated with the staff's participation on a scheduled basis and followed strictly by all laboratory members;
2. Operating procedures (e.g., SOPs monitoring freezer temperatures daily) must be standardized, documented, and supplied to each member of the laboratory staff and updated on a set schedule;
3. Monitoring programs (e.g., surface water monitoring of supplies furnishing public drinking water) must be carefully designed.
4. Maintenance of equipment and instruments must be documented in LIMS or appropriate maintenance books kept with the equipment.
5. Expiration dates of analytical standards, chemicals, and solvents must be observed and replacements made prior to their expiration date.
6. Good laboratory practices (GLPs) must be implemented as needed.
7. Audits must be performed on a scheduled basis to verify that all aspects of the QA program are operating sufficiently.

3.5 QUALITY CONTROL (QC) PROCEDURES

Quality control (QC) concerns procedures that maintain a measurement system in a state of statistical control. This does not mean that statistics control the analytical

procedures but that statistical evidence is used to ensure that the procedure is working under the conditions set by protocol. *The accuracy of an analytical method depends on statistical control being conducted prior to determining any other parameter*. How well the basic method will work with the sample matrix being evaluated will depend on the way the QC samples are examined. A comprehensive QC analytical procedure would include the following:

1. Replicated environmental samples to test the precision of the sampling or analytical procedures.
2. Replicated analyses conducted on the same sample multiple times in order to determine analytical precision.
3. Trip blanks to determine if contaminants are introduced the processes of collecting, shipping, or storing of samples.
4. Matrix-fortified laboratory blanks consisting of solvent and reagent blanks to determine levels of bias due to matrix effects or analytical method problems.
5. Sample blanks (a sample matrix that does not contain the toxicant, although this is sometimes difficult to obtain) to ensure no extraneous or interfering peaks; the peaks indicate where a problem might exist in the method used.
6. Fortified field blanks to determine the effects that the matrix might have on analyte recovery.

3.6 SUMMARY

Planning the essential elements of an analytical protocol is critical to ensuring that the data generated answer what is requested, provide information affecting regulatory action, or enable some decision affecting environmental or human welfare. Essential decision criteria must be included in the protocol that describes the analytical process in detail, including the objective(s) of the study, the QA/QC requirements, the sample plan, methods of analysis, calculations, documentation, and data reporting. Meaningful data can only be generated with the proper method of analysis.

SUGGESTED READING

Statistical Principles

Middlebrooks, E. J. *Statistical Calculations "How to Solve Statistical Problems."* Ann Arbor, MI: Ann Arbor Science Publishers, 1977. (No theory but a lot of practical exercises)

Snedecor, G. W., and W. G. Cochran. 1996 and Numerous editions. *Statistical Methods*. Ames: Iowa State University Press. (One of the classical textbooks on theory and use of statistics)

Steele, R. G. D., and J. H. Torre. 1992 and Numerous editions. *Principles and Procedures of Statistics*. New York: McGraw-Hill. (Another classical textbook)

Youden, W. J., and E. H. Steiner. 1975. Statistical Manual of the AOAC. Arlington, VA: AOAC, 1975. (A presentation of statistical techniques used in collaborative testing of analytical methods)

Environmental Analyses

Currie, L. A. *Detection in Analytical Chemistry: Importance, Theory and Practice*. American Chemical Society (ACS) Symposium Series 361. Washington, DC: ACS, 1988. (Contains both theory and considerable information on detection principles)

Keith, L. H. *Environmental Sampling and Analysis*. Chelsea, MI: Lewis Publishers, 1991.

Quality Assurance

Garfield, F. M. *Quality Assurance Principles for Analytical Laboratories*. Arlington, VA: AOAC, 1984. (An excellent volume containing all phases of QA, including forms, equipment, and audits)

Taylor, J. K. *Quality Assurance of Chemical Measurements*. Chelsea, MI: Lewis Publishers, 1987. (Excellent summary of principles and extensive bibliography)

Analytical Methods

1. University Libraries (many scientific journals contain pesticide residue studies and can be searched through databases available on computer)
   a. Journals include: *Journal of the Association of Official Analytical Chemists* (*JAOAC*); *Journal of Agricultural and Food Chemistry* (*J Agric. Food Chem.*); *Environmental Monitoring and Assessment* (*Environ. Monitoring Assess.*)
   b. Databases include: AGRICOLA, MEDLINE, TOXLINE
2. Official methods, Association of Official Analytical Chemists (AOAC)
3. State and federal agencies and laboratories (e.g., the US EPA repository at Ft. Meade, MD, has many)
4. Web Sites
5. US EPA Methods of Analysis
6. American Society of Testing and Materials (ASTM)
7. Chemical Manufacturers (contact local technical representatives for assistance)