

UNIT 1

GENERAL PRINCIPLES OF TOXICOLOGY

CHAPTER 1

HISTORY AND SCOPE OF TOXICOLOGY

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HISTORY OF TOXICOLOGY

Antiquity

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Toxicology has been defined as the study of the adverse effects of xenobiotics and thus is a borrowing science that has evolved from ancient poisoners. Modern toxicology goes beyond the study of the adverse effects of exogenous agents to the study of molecular biology, using toxicants as tools. Historically, toxicology formed the basis of therapeutics and experimental medicine. Toxicology in this century (1900 to the present) continues to develop and expand by assimilating knowledge and techniques from most branches of biology, chemistry, mathematics, and physics. A recent addition to the field of toxicology (1975 to the present) is the application of the discipline to safety evaluation and risk assessment.

The contributions and activities of toxicologists are diverse and widespread. In the biomedical area, toxicologists are concerned with mechanisms of action and exposure to chemical agents as a cause of acute and chronic illness. Toxicologists contribute to physiology and pharmacology by using toxic agents to understand physiological phenomena. They are involved in the recognition, identification, and quantification of hazards resulting from occupational exposure to chemicals and the public health aspects of chemicals in air, water, other parts of the environment, foods, and drugs. Traditionally, toxicologists have been intimately involved in the discovery and development of new drugs and pesticides. Toxicologists also participate in the development of standards and regulations designed to protect human health and the environment from the adverse effects of chemicals. Environmental toxicologists (a relatively new subset of the discipline) have expanded toxicology to study the effects of chemicals in flora and fauna. Molecular toxicologists are studying the mechanisms by which toxicants modulate cell growth and differentiation and cells respond to toxicants at the level of the gene. In all branches of toxicology, scientists explore the mechanisms by which chemicals produce adverse effects in biological systems. Clinical toxicologists develop antidotes and treatment regimes to ameliorate poisonings and xenobiotic injury. Toxicologists carry out some or all of these activities as members of academic, industrial, and governmental organizations. In doing so, they share methodologies for obtaining data about the toxicity of materials and the responsibility for using this information to make reasonable predictions regarding the hazards of the material to people and the environment. These different but complementary activities characterize the discipline of toxicology.

Toxicology, like medicine, is both a science and an art. The science of toxicology is defined as the observational and data-gathering phase, whereas the art of toxicology consists of the utilization of the data to predict outcomes of exposure in human and animal populations. In most cases, these phases are linked because

the facts generated by the science of toxicology are used to develop extrapolations and hypotheses to explain the adverse effects of chemical agents in situations where there is little or no information. For example, the observation that the administration of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) to female Sprague-Dawley rats induces hepatocellular carcinoma is a fact. However, the conclusion that it will also do so in humans is a prediction or hypothesis. It is important to distinguish facts from predictions. When we fail to distinguish the science from the art, we confuse facts with predictions and argue that they have equal validity, which they clearly do not. In toxicology, as in all sciences, theories have a higher level of certainty than do hypotheses, which in turn are more certain than speculations, opinions, conjectures, and guesses. An insight into modern toxicology and the roles, points of view, and activities of toxicologists can be obtained by examining the historical evolution of the discipline.

HISTORY OF TOXICOLOGY

Antiquity

Toxicology dates back to the earliest humans, who used animal venoms and plant extracts for hunting, warfare, and assassination. The knowledge of these poisons must have predated recorded history. It is safe to assume that prehistoric humans categorized some plants as harmful and others as safe. The same is probably true for the classification of snakes and other animals. The Ebers papyrus (circa 1500 B.C.) contains information pertaining to many recognized poisons, including hemlock (the state poison of the Greeks), aconite (a Chinese arrow poison), opium (used as both a poison and an antidote), and metals such as lead, copper, and antimony. There is also an indication that plants containing substances similar to digitalis and belladonna alkaloids were known. Hippocrates (circa 400 B.C.) added a number of poisons and clinical toxicology principles pertaining to bioavailability in therapy and overdose, while the Book of Job (circa 400 B.C.) speaks of poison arrows (Job 6:4). In the literature of ancient Greece, there are several references to poisons and their use. Some interpretations of Homer have Odysseus obtaining poisons for his arrows (Homer, circa 600 B.C.). Theophrastus (370–286 B.C.), a student of Aristotle, included numerous references to poisonous plants in *De Historia Plantarum*. Dioscorides, a Greek physician in the court of the Roman emperor Nero, made the first attempt at a classification of poisons, which was accompanied by descriptions and drawings. His classification into plant, animal, and mineral poisons not only remained a stan-

dard for 16 centuries but is still a convenient classification (Gunter, 1934). Dioscorides also dabbled in therapy, recognizing the use of emetics in poisoning and the use of caustic agents and cupping glasses in snakebite. Poisoning with plant and animal toxins was quite common. Perhaps the best-known recipient of poison used as a state method of execution was Socrates (470–399 B.C.), whose cup of hemlock extract was apparently estimated to be the proper dose. Expeditious suicide on a voluntary basis also made use of toxicologic knowledge. Demosthenes (385–322 B.C.), who took poison hidden in his pen, was one of many examples. The mode of suicide calling for one to fall on his sword, although manly and noble, carried little appeal and less significance for the women of the day. Cleopatra's (69–30 B.C.) knowledge of natural primitive toxicology permitted her to use the more genteel method of falling on her asp.

The Romans too made considerable use of poisons in politics. One legend tells of King Mithridates VI of Pontus, whose numerous acute toxicity experiments on unfortunate criminals led to his eventual claim that he had discovered an antidote for every venomous reptile and poisonous substance (Guthrie, 1946). Mithridates was so fearful of poisons that he regularly ingested a mixture of 36 ingredients (Galen reports 54) as protection against assassination. On the occasion of his imminent capture by enemies, his attempts to kill himself with poison failed because of his successful antidote concoction, and he was forced to use a sword held by a servant. From this tale comes the term “mithridatic,” referring to an antidotal or protective mixture. The term “theriac” also has become synonymous with “antidote,” although the word comes from the poetic treatise *Theriaca* by Nicander of Colophon (204–135 B.C.), which dealt with poisonous animals; his poem “*Alexipharmaca*” was about antidotes.

Poisonings in Rome reached epidemic proportions during the fourth century B.C. (Livy). It was during this period that a conspiracy of women to remove men from whose death they might profit was uncovered. Similar large-scale poisoning continued until Sulla issued the *Lex Cornelia* (circa 82 B.C.). This appears to be the first law against poisoning, and it later became a regulatory statute directed at careless dispensers of drugs. Nero (A.D. 37–68) used poisons to do away with his stepbrother Britannicus and employed his slaves as food tasters to differentiate edible mushrooms from their more poisonous kin.

Middle Ages

Come bitter pilot, now at once run on
The dashing rocks thy seasick weary bark!
Here's to my love! O true apothecary!
Thy drugs are quick. Thus with a kiss I die.
Romeo and Juliet, act 5, scene 3

Before the Renaissance, the writings of Maimonides (Moses ben Maimon, A.D. 1135–1204) included a treatise on the treatment of poisonings from insects, snakes, and mad dogs (*Poisons and Their Antidotes*, 1198). Maimonides, like Hippocrates before him, wrote on the subject of bioavailability, noting that milk, butter, and cream could delay intestinal absorption. Maimonides also refuted many of the popular remedies of the day and stated his doubts about others. It is rumored that alchemists of this period (circa A.D. 1200), in search of the universal antidote, learned to distill fermented products and made a 60% ethanol beverage that had many interesting powers.

In the early Renaissance, the Italians, with characteristic pragmatism, brought the art of poisoning to its zenith. The poisoner became an integral part of the political scene. The records of the city councils of Florence, particularly those of the infamous Council of Ten of Venice, contain ample testimony about the political use of poisons. Victims were named, prices set, and contracts recorded; when the deed was accomplished, payment was made.

An infamous figure of the time was a lady named Toffana who peddled specially prepared arsenic-containing cosmetics (*Agua Toffana*). Accompanying the product were appropriate instructions for its use. Toffana was succeeded by an imitator with organizational genius, Hieronyma Spara, who provided a new fillip by directing her activities toward specific marital and monetary objectives. A local club was formed of young, wealthy married women, which soon became a club of eligible young wealthy widows, reminiscent of the matronly conspiracy of Rome centuries earlier. Incidentally, arsenic-containing cosmetics were reported to be responsible for deaths well into the twentieth century (Kallett and Schlink, 1933).

Among the prominent families engaged in poisoning, the Borgias were the most notorious. However, many deaths that were attributed to poisoning are now recognized as having resulted from infectious diseases such as malaria. It appears true, however, that Alexander VI, his son Cesare, and Lucrezia Borgia were quite active. The deft application of poisons to men of stature in the Catholic Church swelled the holdings of the papacy, which was their prime heir.

In this period Catherine de Medici exported her skills from Italy to France, where the prime targets of women were their husbands. However, unlike poisoners of an earlier period, the circle represented by Catherine and epitomized by the notorious Marchioness de Brinvilliers depended on developing direct evidence to arrive at the most effective compounds for their purposes. Under the guise of delivering provender to the sick and the poor, Catherine tested toxic concoctions, carefully noting the rapidity of the toxic response (onset of action), the effectiveness of the compound (potency), the degree of response of the parts of the body (specificity, site of action), and the complaints of the victim (clinical signs and symptoms).

The culmination of the practice in France is represented by the commercialization of the service by Catherine Deshayes, who earned the title “La Voisine.” Her business was dissolved by her execution. Her trial was one of the most famous of those held by the *Chambre Ardente*, a special judicial commission established by Louis XIV to try such cases without regard to age, sex, or national origin. La Voisine was convicted of many poisonings, with over 2000 infants among her victims.

Age of Enlightenment

All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy.

Paracelsus

A significant figure in the history of science and medicine in the late Middle Ages was the renaissance man Philippus Aureolus Theophrastus Bombastus von Hohenheim-Paracelsus (1493–1541). Between the time of Aristotle and the age of Paracelsus, there was little substantial change in the biomedical sciences. In the sixteenth century, the revolt against the authority of the Catholic Church was accompanied by a parallel attack on the godlike au-

thority exercised by the followers of Hippocrates and Galen. Paracelsus personally and professionally embodied the qualities that forced numerous changes in this period. He and his age were pivotal, standing between the philosophy and magic of classical antiquity and the philosophy and science willed to us by figures of the seventeenth and eighteenth centuries. Clearly, one can identify in Paracelsus's approach, point of view, and breadth of interest numerous similarities to the discipline that is now called toxicology.

Paracelsus, a physician-chemist and the son of a physician, formulated many revolutionary views that remain an integral part of the structure of toxicology, pharmacology, and therapeutics today (Pagel, 1958). He promoted a focus on the "toxicon," the primary toxic agent, as a chemical entity, as opposed to the Grecian concept of the mixture or blend. A view initiated by Paracelsus that became a lasting contribution held as corollaries that (1) experimentation is essential in the examination of responses to chemicals, (2) one should make a distinction between the therapeutic and toxic properties of chemicals, (3) these properties are sometimes but not always indistinguishable except by dose, and (4) one can ascertain a degree of specificity of chemicals and their therapeutic or toxic effects. These principles led Paracelsus to introduce mercury as the drug of choice for the treatment of syphilis, a practice that survived 300 years but led to his famous trial. This viewpoint presaged the "magic bullet" (arsphenamine) of Paul Ehrlich and the introduction of the therapeutic index. Further, in a very real sense, this was the first sound articulation of the dose-response relation, a bulwark of toxicology (Pachter, 1961).

The tradition of the poisoners spread throughout Europe, and their deeds played a major role in the distribution of political power throughout the Middle Ages. Pharmacology as it is known today had its beginnings during the Middle Ages and early Renaissance. Concurrently, the study of the toxicity and the dose-response relationship of therapeutic agents was commencing.

The occupational hazards associated with metalworking were recognized during the fifteenth century. Early publications by Ellenbog (circa 1480) warned of the toxicity of the mercury and lead exposures involved in goldsmithing. Agricola published a short treatise on mining diseases in 1556. However, the major work on the subject, *On the Miners' Sickness and Other Diseases of Miners* (1567), was published by Paracelsus. This treatise addressed the etiology of miners' disease, along with treatment and prevention strategies. Occupational toxicology was further advanced by the work of Bernardino Ramazzini. His classic, published in 1700 and entitled *Discourse on the Diseases of Workers*, set the standard for occupational medicine well into the nineteenth century. Ramazzini's work broadened the field by discussing occupations ranging from miners to midwives and including printers, weavers, and potters.

The developments of the industrial revolution stimulated a rise in many occupational diseases. Percival Pott's (1775) recognition of the role of soot in scrotal cancer among chimney sweeps was the first reported example of polyaromatic hydrocarbon carcinogenicity, a problem that still plagues toxicologists today. These findings led to improved medical practices, particularly in prevention. It should be noted that Paracelsus and Ramazzini also pointed out the toxicity of smoke and soot.

The nineteenth century dawned in a climate of industrial and political revolution. Organic chemistry was in its infancy in 1800, but by 1825 phosgene (COCl_2) and mustard gas (bis[2-chloroethyl]sulfide) had been synthesized. These two agents were

used in World War I as war gases. By 1880 over 10,000 organic compounds had been synthesized including chloroform, carbon tetrachloride, diethyl ether, and carbonic acid, and petroleum and coal gasification by-products were used in trade (Zapp, 1982). Determination of the toxicologic potential of these newly created chemicals became the underpinning of the science of toxicology as it is practiced today. However, there was little interest during the mid-nineteenth century in hampering industrial development. Hence, the impact of industrial toxicology discoveries was not felt until the passage of worker's insurance laws, first in Germany (1883), then in England (1897), and later in the United States (1910).

Experimental toxicology accompanied the growth of organic chemistry and developed rapidly during the nineteenth century. Magendie (1783–1885), Orfila (1787–1853), and Bernard (1813–1878) carried out truly seminal research in experimental toxicology and laid the groundwork for pharmacology and experimental therapeutics as well as occupational toxicology.

Orfila, a Spanish physician in the French court, was the first toxicologist to use autopsy material and chemical analysis systematically as legal proof of poisoning. His introduction of this detailed type of analysis survives as the underpinning of forensic toxicology (Orfila, 1818). Orfila published the first major work devoted expressly to the toxicity of natural agents (1815). Magendie, a physician and experimental physiologist, studied the mechanisms of action of emetine, strychnine, and "arrow poisons" (Olmsted, 1944). His research into the absorption and distribution of these compounds in the body remains a classic in toxicology and pharmacology. One of Magendie's more famous students, Claude Bernard, continued the study of arrow poisons (Bernard, 1850) but also added works on the mechanism of action of carbon monoxide. Bernard's treatise, *An Introduction to the Study of Experimental Medicine* (translated by Greene in 1949), is a classic in the development of toxicology.

Many German scientists contributed greatly to the growth of toxicology in the late nineteenth and early twentieth centuries. Among the giants of the field are Oswald Schmiedeberg (1838–1921) and Louis Lewin (1850–1929). Schmiedeberg made many contributions to the science of toxicology, not the least of which was the training of approximately 120 students who later populated the most important laboratories of pharmacology and toxicology throughout the world. His research focused on the synthesis of hippuric acid in the liver and the detoxification mechanisms of the liver in several animal species (Schmiedeberg and Koppe, 1869). Lewin, who was educated originally in medicine and the natural sciences, trained in toxicology under Liebreich at the Pharmacological Institute of Berlin (1881). His contributions on the chronic toxicity of narcotics and other alkaloids remain a classic. Lewin also published much of the early work on the toxicity of methanol, glycerol, acrolein, and chloroform (Lewin, 1920, 1929).

MODERN TOXICOLOGY

Toxicology has evolved rapidly during this century. The exponential growth of the discipline can be traced to the World War II era with its marked increase in the production of drugs, pesticides, munitions, synthetic fibers, and industrial chemicals. The history of many sciences represents an orderly transition based on theory, hypothesis testing, and synthesis of new ideas. Toxicology, as a gathering and an applied science, has, by contrast, developed in fits and

starts. Toxicology calls on almost all the basic sciences to test its hypotheses. This fact, coupled with the health and occupational regulations that have driven toxicology research since 1900, has made this discipline exceptional in the history of science. The differentiation of toxicology as an art and a science, though arbitrary, permits the presentation of historical highlights along two major lines.

Modern toxicology can be viewed as a continuation of the development of the biological and physical sciences in the late nineteenth and twentieth centuries (Table 1-1). During the second half of the nineteenth century, the world witnessed an explosion in science that produced the beginning of the modern era of medicine, synthetic chemistry, physics, and biology. Toxicology has drawn its strength and diversity from its proclivity to borrowing. With the advent of anesthetics and disinfectants and the advancement of experimental pharmacology in the late 1850s, toxicology as it is currently understood got its start. The introduction of ether, chloro-

form, and carbonic acid led to several iatrogenic deaths. These unfortunate outcomes spurred research into the causes of the deaths and early experiments on the physiological mechanisms by which these compounds caused both beneficial and adverse effects. By the late nineteenth century the use of organic chemicals was becoming more widespread, and benzene, toluene, and the xylenes went into larger-scale commercial production.

During this period, the use of “patent” medicines was prevalent, and there were several incidents of poisonings from these medicaments. The adverse reactions to patent medicines, coupled with the response to Upton Sinclair’s exposé of the meat-packing industry in *The Jungle*, culminated in the passage of the Wiley Bill (1906), the first of many U.S. pure food and drug laws (see Hutt and Hutt, 1984, for regulatory history).

A working hypothesis about the development of toxicology is that the discipline expands in response to legislation, which itself is a response to a real or perceived tragedy. The Wiley bill was the

Table 1-1
Selection of Developments in Toxicology

<i>Development of early advances in analytic methods</i>	
Marsh, 1836:	development of method for arsenic analysis
Reinsh, 1841:	combined method for separation and analysis of As and Hg
Fresenius, 1845, and von Babo, 1847:	development of screening method for general poisons
Stas-Otto, 1851:	extraction and separation of alkaloids
Mitscherlich, 1855:	detection and identification of phosphorus
<i>Early mechanistic studies</i>	
F. Magendie, 1809:	study of “arrow poisons,” mechanism of action of emetine and strychnine
C. Bernard, 1850:	carbon monoxide combination with hemoglobin, study of mechanism of action of strychnine, site of action of curare
R. Bohm, ca. 1890:	active anthelmintics from fern, action of croton oil catharsis, poisonous mushrooms
<i>Introduction of new toxicants and antidotes</i>	
R. A. Peters, L. A. Stocken, and R. H. S. Thompson, 1945:	development of British Anti Lewisite (BAL) as a relatively specific antidote for arsenic, toxicity of monofluorocarbon compounds
K. K. Chen, 1934:	introduction of modern antidotes (nitrite and thiosulfate) for cyanide toxicity
C. Voegtlin, 1923:	mechanism of action of As and other metals on the SH groups
P. Müller, 1944–1946:	introduction and study of DDT (dichlorodiphenyltrichloroethane) and related insecticide compounds
G. Schrader, 1952:	introduction and study of organophosphorus compounds
R. N. Chopra, 1933:	indigenous drugs of India
<i>Miscellaneous toxicologic studies</i>	
R. T. Williams:	study of detoxication mechanisms and species variation
A. Rothstein:	effects of uranium ion on cell membrane transport
R. A. Kehoe:	investigation of acute and chronic effects of lead
A. Vorwald:	studies of chronic respiratory disease (beryllium)
H. Hardy:	community and industrial poisoning (beryllium)
A. Hamilton:	introduction of modern industrial toxicology
H. C. Hodge:	toxicology of uranium, fluorides; standards of toxicity
A. Hoffman:	introduction of lysergic acid and derivatives; psychotomimetics
R. A. Peters:	biochemical lesions, lethal synthesis
A. E. Garrod:	inborn errors of metabolism
T. T. Litchfield and F. Wilcoxon:	simplified dose-response evaluation
C. J. Bliss:	method of probits, calculation of dosage-mortality curves

first such reaction in the area of food and drugs, and the worker's compensation laws cited above were a response to occupational toxicities. In addition, the National Safety Council was established in 1911, and the Division of Industrial Hygiene was established by the U.S. Public Health Service in 1914. A corollary to this hypothesis might be that the founding of scientific journals and/or societies is sparked by the development of a new field. The *Journal of Industrial Hygiene* began in 1918. The major chemical manufacturers in the United States (Dow, Union Carbide, and Du Pont) established internal toxicology research laboratories to help guide decisions on worker health and product safety.

During the 1890s and early 1900s, the French scientists Becquerel and the Curies reported the discovery of "radioactivity." This opened up for exploration a very large area in physics, biology, and medicine, but it would not actively affect the science of toxicology for another 40 years. However, another discovery, that of vitamins, or "vital amines," was to lead to the use of the first large-scale bioassays (multiple animal studies) to determine whether these "new" chemicals were beneficial or harmful to laboratory animals. The initial work in this area took place at around the time of World War I in several laboratories, including the laboratory of Philip B. Hawk in Philadelphia. Hawk and a young associate, Bernard L. Oser, were responsible for the development and verification of many early toxicologic assays that are still used in a slightly amended form. Oser's contributions to food and regulatory toxicology were extraordinary. These early bioassays were made possible by a major advance in toxicology: the availability of developed and refined strains of inbred laboratory rodents (Donaldson, 1912).

The 1920s saw many events that began to mold the fledgling field of toxicology. The use of arsenicals for the treatment of diseases such as syphilis (arsenicals had been used in agriculture since the mid-nineteenth century) resulted in acute and chronic toxicity. Prohibition of alcoholic beverages in the United States opened the door for early studies of neurotoxicology, with the discovery that triorthocresyl phosphate (TOCP), methanol, and lead (all products of "bootleg" liquor) are neurotoxicants. TOCP, which is a modern gasoline additive, caused a syndrome that became known as "ginger-jake" walk, a spastic gait resulting from drinking adulterated ginger beer. Mueller's discovery of DDT (dichlorodiphenyl-trichloroethane) and several other organohalides, such as hexachlorobenzene and hexachlorocyclohexane, during the late 1920s resulted in wider use of insecticidal agents. Other scientists were hard at work attempting to elucidate the structures and activity of the estrogens and androgens. Work on the steroid hormones led to the use of several assays for the determination of the biological activity of organ extracts and synthetic compounds. Efforts to synthesize steroid-like chemicals were spearheaded by E. C. Dodds and his coworkers, one of whom was Leon Golberg, a young organic chemist. Dodds's work on the bioactivity of the estrogenic compounds resulted in the synthesis of diethylstilbestrol (DES), hexestrol, and other stilbenes and the discovery of the strong estrogenic activity of substituted stilbenes. Golberg's intimate involvement in this work stimulated his interest in biology, leading to degrees in biochemistry and medicine and a career in toxicology in which he oversaw the creation of the laboratories of the British Industrial Biological Research Association (BIBRA) and the Chemical Industry Institute of Toxicology (CIIT). Interestingly, the initial observations that led to the discovery of DES were the findings of feminization of animals treated with the experimental carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA).

The 1930s saw the world preparing for World War II and a major effort by the pharmaceutical industry in Germany and the United States to manufacture the first mass-produced antibiotics. One of the first journals expressly dedicated to experimental toxicology, *Archiv für Toxikologie*, began publication in Europe in 1930, the same year that Herbert Hoover signed the act that established the National Institutes of Health (NIH) in the United States.

The discovery of sulfanilamide was heralded as a major event in combating bacterial diseases. However, for a drug to be effective, there must be a reasonable delivery system, and sulfanilamide is highly insoluble in an aqueous medium. Therefore, it was originally prepared in ethanol (elixir). However, it was soon discovered that the drug was more soluble in ethylene glycol, which is a dihydroxy rather than a monohydroxy ethane. The drug was sold in glycol solutions but was labeled as an elixir, and several patients died of acute kidney failure resulting from the metabolism of the glycol to oxalic acid and glycolic acid, with the acids, along with the active drug, crystallizing in the kidney tubules. This tragic event led to the passage of the Copeland bill in 1938, the second major bill involving the formation of the U.S. Food and Drug Administration (FDA). The sulfanilamide disaster played a critical role in the further development of toxicology, resulting in work by Eugene Maximilian Geiling in the Pharmacology Department of the University of Chicago that elucidated the mechanism of toxicity of both sulfanilamide and ethylene glycol. Studies of the glycols were simultaneously carried out at the U.S. FDA by a group led by Arnold Lehman. The scientists associated with Lehman and Geiling were to become the leaders of toxicology over the next 40 years. With few exceptions, toxicology in the United States owes its heritage to Geiling's innovativeness and ability to stimulate and direct young scientists and Lehman's vision of the use of experimental toxicology in public health decision making. Because of Geiling's reputation, the U.S. government turned to this group for help in the war effort. There were three main areas in which the Chicago group took part during World War II: the toxicology and pharmacology of organophosphate chemicals, antimalarial drugs, and radionuclides. Each of these areas produced teams of toxicologists who became academic, governmental, and industrial leaders in the field.

It was also during this time that DDT and the phenoxy herbicides were developed for increased food production and, in the case of DDT, control of insect-borne diseases. These efforts between 1940 and 1946 led to an explosion in toxicology. Thus, in line with the hypothesis advanced above, the crisis of World War II caused the next major leap in the development of toxicology.

If one traces the history of the toxicology of metals over the past 45 years, the role of the Chicago group is quite visible. This story commences with the use of uranium for the "bomb" and continues today with research on the role of metals in their interactions with DNA, RNA, and growth factors. Indeed, the Manhattan Project created a fertile environment that resulted in the initiation of quantitative biology, radiotracer technology, and inhalation toxicology. These innovations have revolutionized modern biology, chemistry, therapeutics, and toxicology.

Inhalation toxicology began at the University of Rochester under the direction of Stafford Warren, who headed the Department of Radiology. He developed a program with colleagues such as Harold Hodge (pharmacologist), Herb Stokinger (chemist), Sid Laskin (inhalation toxicologist), and Lou and George Casarett (toxicologists). These young scientists were to go on to become giants

in the field. The other sites for the study of radionuclides were Chicago for the “internal” effects of radioactivity and Oak Ridge, Tennessee, for the effects of “external” radiation. The work of the scientists on these teams gave the scientific community data that contributed to the early understanding of macromolecular binding of xenobiotics, cellular mutational events, methods for inhalation toxicology and therapy, and toxicological properties of trace metals, along with a better appreciation of the complexities of the dose-response curve.

Another seminal event in toxicology that occurred during the World War II era was the discovery of organophosphate cholinesterase inhibitors. This class of chemicals, which was discovered by Willy Lange and Gerhard Schrader, was destined to become a driving force in the study of neurophysiology and toxicology for several decades. Again, the scientists in Chicago played major roles in elucidating the mechanisms of action of this new class of compounds. Geiling’s group, Kenneth Dubois in particular, were leaders in this area of toxicology and pharmacology. Dubois’s students, particularly Sheldon Murphy, continued to be in the forefront of this special area. The importance of the early research on the organophosphates has taken on special meaning in the years since 1960, when these nonbioaccumulating insecticides were destined to replace DDT and other organochlorine insecticides.

Early in the twentieth century, it was demonstrated experimentally that quinine has a marked effect on the malaria parasite [it had been known for centuries that chincona bark extract is efficacious for “Jesuit fever” (malaria)]. This discovery led to the development of quinine derivatives for the treatment of the disease and the formulation of the early principles of chemotherapy. The pharmacology department at Chicago was charged with the development of antimalarials for the war effort. The original protocols called for testing of efficacy and toxicity in rodents and perhaps dogs and then the testing of efficacy in human volunteers. One of the investigators charged with generating the data needed to move a candidate drug from animals to humans was Fredrick Coulston. This young parasitologist and his colleagues, working under Geiling, were to evaluate potential drugs in animal models and then establish human clinical trials. It was during these experiments that the use of nonhuman primates came into vogue for toxicology testing. It had been noted by Russian scientists that some antimalarial compounds caused retinopathies in humans but did not apparently have the same adverse effect in rodents and dogs. This finding led the Chicago team to add one more step in the development process: toxicity testing in rhesus monkeys just before efficacy studies in people. This resulted in the prevention of blindness in untold numbers of volunteers and perhaps some of the troops in the field. It also led to the school of thought that nonhuman primates may be one of the better models for humans and the establishment of primate colonies for the study of toxicity. Coulston pioneered this area of toxicology and remains committed to it.

Another area not traditionally thought of as toxicology but one that evolved during the 1940s as an exciting and innovative field is experimental pathology. This branch of experimental biology developed from bioassays of estrogens and early experiments in chemical- and radiation-induced carcinogenesis. It is from these early studies that hypotheses on tumor promotion and cancer progression have evolved.

Toxicologists today owe a great deal to the researchers of chemical carcinogenesis of the 1940s. Much of today’s work can

be traced to Elizabeth and James Miller at Wisconsin. This husband and wife team started under the mentorship of Professor Rusch, the director of the newly formed McArdle Laboratory for Cancer Research, and Professor Baumann. The seminal research of the Millers led to the discovery of the role of reactive intermediates in carcinogenicity and that of mixed-function oxidases in the endoplasmic reticulum. These findings, which initiated the great works on the cytochrome-P450 family of proteins, were aided by two other major discoveries for which toxicologists (and all other biological scientists) are deeply indebted: paper chromatography in 1944 and the use of radiolabeled dibenzanthracene in 1948. Other major events of note in drug metabolism included the work of Bernard Brodie on the metabolism of methyl orange in 1947. This piece of seminal research led to the examination of blood and urine for chemical and drug metabolites. It became the tool with which one could study the relationship between blood levels and biological action. The classic treatise of R. T. Williams, *Detoxication Mechanisms*, was published in 1947. This text described the many pathways and possible mechanisms of detoxication and opened the field to several new areas of study.

The decade after World War II was not as boisterous as the period from 1935 to 1945. The first major U.S. pesticide act was signed into law in 1947. The significance of the initial Federal Insecticide, Fungicide, and Rodenticide Act was that for the first time in U.S. history a substance that was neither a drug nor a food had to be shown to be safe and efficacious. This decade, which coincided with the Eisenhower years, saw the dispersion of the groups from Chicago, Rochester, and Oak Ridge and the establishment of new centers of research. Adrian Albert’s classic *Selective Toxicity* was published in 1951. This treatise, which has appeared in several editions, presented a concise documentation of the principles of the site-specific action of chemicals.

AFTER WORLD WAR II

You too can be a toxicologist in two easy lessons, each of ten years.

Arnold Lehman (circa 1955)

The mid-1950s witnessed the strengthening of the U.S. Food and Drug Administration’s commitment to toxicology under the guidance of Arnold Lehman. Lehman’s tutelage and influence are still felt today. The adage “You too can be a toxicologist” is as important a summation of toxicology as the often quoted statement of Paracelsus: “The dose makes the poison.” The period from 1955 to 1958 produced two major events that would have a long-lasting impact on toxicology as a science and a professional discipline. Lehman, Fitzhugh, and their coworkers formalized the experimental program for the appraisal of food, drug, and cosmetic safety in 1955, updated by the U.S. FDA in 1982, and the Gordon Research Conferences established a conference on toxicology and safety evaluation, with Bernard L. Oser as its initial chairman. These two events led to close relationships among toxicologists from several groups and brought toxicology into a new phase. At about the same time, the U.S. Congress passed and the president of the United States signed the additives amendments to the Food, Drug, and Cosmetic Act. The Delaney clause (1958) of these amendments stated broadly that any chemical found to be carcinogenic in laboratory animals or humans could not be added to the U.S. food supply. The impact of this legislation cannot be overstated. Delaney became a battle cry for many groups and resulted

in the inclusion at a new level of biostatisticians and mathematical modelers in the field of toxicology. It fostered the expansion of quantitative methods in toxicology and led to innumerable arguments about the “one-hit” theory of carcinogenesis. Regardless of one’s view of Delaney, it has served as an excellent starting point for understanding the complexity of the biological phenomenon of carcinogenicity and the development of risk assessment models. One must remember that at the time of Delaney, the analytic detection level for most chemicals was 20 to 100 ppm (today, parts per quadrillion). Interestingly, the Delaney clause has been invoked only on a few occasions, and it has been stated that Congress added little to the food and drug law with this clause (Hutt and Hutt, 1984).

Shortly after the Delaney amendment and after three successful Gordon Conferences, the first American journal dedicated to toxicology was launched by Coulston, Lehman, and Hayes. *Toxicology and Applied Pharmacology* has been the flagship journal of toxicology ever since. The founding of the Society of Toxicology followed shortly afterward, and this journal became its official publication. The society’s founding members were Fredrick Coulston, William Deichmann, Kenneth DuBois, Victor Drill, Harry Hayes, Harold Hodge, Paul Larson, Arnold Lehman, and C. Boyd Shaffer. These researchers deserve a great deal of credit for the growth of toxicology. DuBois and Geiling published their *Textbook of Toxicology* in 1959.

The 1960s were a tumultuous time for society, and toxicology was swept up in the tide. Starting with the tragic thalidomide incident, in which several thousand children were born with serious birth defects, and the publication of Rachel Carson’s *Silent Spring* (1962), the field of toxicology developed at a feverish pitch. Attempts to understand the effects of chemicals on the embryo and fetus and on the environment as a whole gained momentum. New legislation was passed, and new journals were founded. The education of toxicologists spread from the deep traditions at Chicago and Rochester to Harvard, Miami, Albany, Iowa, Jefferson, and beyond. Geiling’s fledglings spread as Schmiedeberg’s had a half century before. Many new fields were influencing and being assimilated into the broad scope of toxicology, including environmental sciences, aquatic and avian biology, cell biology, analytic chemistry, and genetics.

During the 1960s, particularly the latter half of the decade, the analytic tools used in toxicology were developed to a level of sophistication that allowed the detection of chemicals in tissues and other substrates at part per billion concentrations (today parts per quadrillion may be detected). Pioneering work in the development of point mutation assays that were replicable, quick, and inexpensive led to a better understanding of the genetic mechanisms of carcinogenicity (Ames, 1983). The combined work of Ames and the Millers (Elizabeth C. and James A.) at McArdle Laboratory allowed the toxicology community to make major contributions to the understanding of the carcinogenic process.

The low levels of detection of chemicals and the ability to detect point mutations rapidly created several problems and opportunities for toxicologists and risk assessors that stemmed from interpretation of the Delaney amendment. Cellular and molecular toxicology developed as a subdiscipline, and risk assessment became a major product of toxicological investigations.

The establishment of the National Center for Toxicologic Research (NCTR), the expansion of the role of the U.S. FDA, and the establishment of the U.S. Environmental Protection Agency

(EPA) and the National Institute of Environmental Health Sciences (NIEHS) were considered clear messages that the government had taken a strong interest in toxicology. Several new journals appeared during the 1960s, and new legislation was written quickly after *Silent Spring* and the thalidomide disaster.

The end of the 1960s witnessed the “discovery” of TCDD as a contaminant in the herbicide Agent Orange (the original discovery of TCDD toxicity was reported in 1957). The research on the toxicity of this compound has produced some very good and some very poor research in the field of toxicology. The discovery of a high-affinity cellular binding protein designated the “Ah” receptor (see Poland and Knutsen, 1982, for a review) at the McArdle Laboratory and work on the genetics of the receptor at NIH (Nebert and Gonzalez, 1987) have revolutionized the field of toxicology. The importance of TCDD to toxicology lies in the fact that it forced researchers, regulators, and the legal community to look at the role of mechanisms of toxic action in a different fashion.

At least one other event precipitated a great deal of legislation during the 1970s: Love Canal. The “discovery” of Love Canal led to major concerns regarding hazardous wastes, chemical dump sites, and disclosure of information about those sites. Soon after Love Canal, the EPA listed several equally contaminated sites in the United States. The agency was given the responsibility to develop risk assessment methodology to determine health risks from exposure to effluents and to attempt to remediate these sites. These combined efforts led to broad-based support for research into the mechanisms of action of individual chemicals and complex mixtures. Love Canal and similar issues created the legislative environment that led to the Toxic Substances Control Act and eventually to the Superfund bill. These omnibus bills were created to cover the toxicology of chemicals from initial synthesis to disposal (cradle to grave).

The expansion of legislation, journals, and new societies involved with toxicology was exponential during the 1970s and 1980s and shows no signs of slowing down. Currently, in the United States there are dozens of professional, governmental, and other scientific organizations with thousands of members and over 120 journals dedicated to toxicology and related disciplines.

In addition, toxicology continues to expand in stature and in the number of programs worldwide. The International Congress of Toxicology is made up of toxicology societies from Europe, South America, Asia, Africa, and Australia and brings together the broadest representation of toxicologists.

The original Gordon Conference series has changed to Mechanisms of Toxicity, and several other conferences related to special areas of toxicology are now in existence. The American Society of Toxicology has formed specialty sections and regional chapters to accommodate the over 5000 scientists involved in toxicology today. Texts and reference books for toxicology students and scientists abound. Toxicology has evolved from a borrowing science to a seminal discipline seeding the growth and development of several related fields of science and science policy.

The history of toxicology has been interesting and varied but never dull. Perhaps as a science that has grown and prospered by borrowing from many disciplines, it has suffered from the absence of a single goal, but its diversification has allowed for the interspersing of ideas and concepts from higher education, industry, and government. As an example of this diversification, one now finds toxicology graduate programs in medical schools, schools of pub-

lic health, and schools of pharmacy as well as programs in environmental science and engineering and undergraduate programs in toxicology at several institutions. Surprisingly, courses in toxicology are now being offered in several liberal arts undergraduate schools as part of their biology and chemistry curricula. This has

resulted in an exciting, innovative, and diversified field that is serving science and the community at large.

Few disciplines can point to both basic sciences and direct applications at the same time. Toxicology—the study of the adverse effects of xenobiotics—may be unique in this regard.

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