Forensic Toxicology

Principles and Concepts

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For Marcia,
wife and mother extraordinaire,
with gratitude

Preface

In the preface to their 1981 book *Introduction to Forensic Toxicology*, editors Robert H. Cravey and Randall C. Baselt stated that it was their opinion that up until 1975 "… the only presentations of modern forensic toxicology that could be used for teaching purposes were an 18-page chapter by C.P. Stewart and A. Stolman entitled *The toxicologist and his work* in their book *Toxicology: Mechanisms and Analytical Methods* (1960) and the first two chapters from A.S. Curry's *Poison Detection in Human Organs* (1963)." For one of us who began teaching forensic toxicology at the graduate level in 1975, this lack of textual material suitable for beginning students in forensic toxicology was readily apparent. A great deal of the original literature consisted of case reports, which, although important for practitioners, did not provide students with the principles and concepts that they required.

In the last quarter of the twentieth century and the first years of the twenty-first century, there has been a dramatic increase (an explosion) in the literature of forensic toxicology—journals and books have proliferated. There are several reasons for this upsurge, including rapid advances in methods of analyses, an improved understanding of the interpretation of postmortem and antemortem analytical results, and a better understanding of problems specific to forensic toxicologists, such as postmortem redistribution and factors influencing drug stability.

As significant and important as the advances in the literature of forensic toxicology have been, there has been relatively little literature, other than review articles and portions of a few books, suitable for students and professionals beginning their study of forensic toxicology. Many books on the subject attempt to cover the entire topic in a single volume, incorporating the theory of instrumental methods and immunological analysis, drug disposition, mechanisms of drug action, therapeutic and adverse drug effects (including pathological findings), postmortem analysis, and interpretation as well as chapters on individual drugs of abuse. We are of the opinion that a text suitable for the beginner should introduce the fundamental principles and concepts of forensic toxicology, which introductory texts in forensic toxicology often do not cover adequately. The details of instrumental theory and practice and the toxicology of abused drugs often are included at the expense of the foundational principles of toxicology.

The content in *Forensic Toxicology: Principles and Concepts* is based upon two graduate courses in forensic toxicology that one of us has taught for 40 years to hundreds of master's degree candidates in forensic sciences at The George Washington University. The text is not meant to be encyclopedic in nature, but rather to provide an overview of the largely unchanging core tenets of the discipline: analysis, interpretation, and reporting.

We hope that *Forensic Toxicology: Principles and Concepts* will serve as a core resource not only for upper-level undergraduate students and beginning graduate students studying forensic toxicology and/or forensic chemistry, but also for scientists who are beginning their careers in forensic toxicology laboratories.

We have chosen to focus on topics that beginning toxicology students generally will not have been exposed to previously. As such, our text does not include theories of instrumental methods of analysis, the knowledge of which, although of paramount importance, is common to most beginning students in forensic toxicology who are, or were, undergraduate chemistry majors. These topics are excluded not only because a familiarity with these topics has often been obtained previously by students, but also because they are dealt with in great detail in numerous other excellent sources. However, since these students generally do not have experience with certain foundational subjects important to forensic toxicologists, including pharmacokinetics, pharmacodynamics, immunology, and toxicogenomics, appendices introducing these topics have been included. In addition, an appendix containing a review of selected cases in which the core principles of toxicology were applied is included.

The text contains the following chapters:

Chapter 1, The Development of Forensic Toxicology is an introduction to the discipline with an emphasis on the founding scientists and historical landmarks demonstrating that roughly 200 years ago, the creators of this discipline not only identified problems unique to the field, but also established many of the principles that continue to be employed in modern forensic toxicology.

Chapter 2, The Duties and Responsibilities of Forensic Toxicologists is a summary of the core professional activities of forensic toxicologists—analysis, interpretation, and reporting—each of which is the topic of an entire unit in the book and will be presented in greater detail in the chapters of those units.

Chapter 3, Forensic Toxicology Resources identifies a number of the books, journals, online resources, and organizations from which information of direct or peripheral importance to forensic toxicology may be found.

Chapter 4, The Laboratory examines the administration and functions of a modern forensic toxicology laboratory.

Chapter 5, Analytical Strategy describes the various protocols employed by forensic toxicology laboratories for the detection of drugs in biological samples.

Chapter 6, Sample Handling focuses on the principles underlying the selection, collection, preservation, and transmittal of samples to the laboratory prior to their analysis.

Chapter 7, Storage Stability of Analytes describes the factors that may influence analyte stability in stored samples and provides an overview of the strategies commonly utilized to maximize analyte stability.

Chapter 8, Analytical Samples considers the common and uncommon samples analyzed by forensic toxicologists, including the merits and disadvantages of each.

Chapter 9, Sample Preparation provides an overview of the methods of sample preparation that are most commonly utilized in forensic toxicology laboratories.

Chapter 10, Methods of Detection, Identification, and Quantitation provides an overview of the criteria that should be utilized for selecting a method of analysis, with a focus on the benefits and disadvantages, as well as the sources of error, of several of the methods that are widely employed in forensic toxicology laboratories.

Chapter 11, Quality Assurance and Quality Control describes the components of a quality assurance/quality control program in a forensic toxicology laboratory.

Chapter 12, Types of Interpretations assesses the opinions that can and cannot be made based on analytical results and identifies those factors that may affect the conclusions drawn by forensic toxicologists.

Chapter 13, Reports is a description of the information that should be included in official reports of analytical toxicology results and an overview of the manner by which written reports should be prepared.

Chapter 14, Testifying is a description of the process of giving sworn testimony at deposition or in court. The role of the expert at trial, the preparation for and manner of providing expert testimony, including a presentation of the "shoulds" and "should nots" of testifying, are presented.

Appendix A, Principles of Pharmacokinetics is a presentation of the theories of drug absorption, distribution, metabolism, and excretion, emphasizing those that are of particular importance to forensic toxicologists.

Appendix B, Principles of Pharmacodynamics considers the mechanisms of drug action that are important to interpretations made in forensic toxicology.

Appendix C, Immunoassays explains those aspects of immunology that are of importance to forensic toxicologists, including an overview of the immune system and the theory of immunoassays.

Appendix D, Toxicogenomics examines the effects of genetic differences on pharmacokinetics and pharmacodynamics and describes how genetic polymorphisms may affect the interpretation of analytical results.

Appendix E, Famous Cases in Forensic Toxicology is a presentation of specific cases in which forensic toxicology played an important role.

In reviewing the literature for the preparation of this book, we have been impressed by the intelligence, insights, and intellectual power that so many forensic toxicologists, past and present, have brought to their work and as a result, to the development of forensic toxicology. We are appreciative of their efforts and we hope that we have represented their work accurately.

We are grateful also to our students. As is common for teachers, we have learned far more from our students than they have learned from us. As it is true that the dose makes the poison, it is also true that the students make the teacher: for this we are thankful to our many students.

Nicholas T. Lappas
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CHAPTER

1 The Development of Forensic Toxicology

Of all of the branches of Medicine, the study of Toxicology is without contradiction that which excites the most general interest.
Mathieu Joseph Bonaventure Orfila

1.1 DEFINITIONS

1.1.1 TOXICOLOGY

The word "toxicology" stems from the Indo-European root word *tekw*, meaning to flee or run from which are derived the Greek *toxon*, bow, and *the* Latin, *toxicum*, poison (McKean, 2005).

Many definitions of toxicology have been proposed, but generally all emphasize that toxicology is the study of adverse effects produced by drugs and chemicals.

- "Toxicology is the study of the harmful actions of chemicals on biologic tissue" (Loomis and Hayes, 1996).
- "Toxicology is the study of the adverse effects of chemical or physical agents on biological systems: it is the science of poisons" (Hayes, 2001).
- "Toxicology is concerned with the deleterious effects of these chemical agents on all living systems" (Plaa, 2007).
- "Toxicology is the study of the adverse effects of chemicals on living organisms" (Eaton and Klaassen, 2001).
- "Toxicology is the study of the adverse effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects" (Society of Toxicology, 2005).
- "Toxicology is the science of poisons including their sources, chemical composition, actions, tests and antidotes their nature effects and antibodies" (Stedman's medical dictionary, 2006).

1.1.2 POISON

The word "poison" is the same as the Old French word for magic potion, which stems from the Latin, *potare*, to drink (McKean, 2005). The use of the word "poison" to describe chemicals that cause adverse effects is problematic since it implies that there exist substances that produce ***only*** adverse effects regardless of the conditions

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of exposure—a concept discarded by Paracelsus almost 500 years ago (see below). Unfortunately, the word poisons is used in the title of the standard one-volume toxicology text, *Toxicology: the Basic Science of Poisons*. We will attempt to refrain from the use of the word "poison" in this text as it is now known that all chemicals can produce serious adverse effects if administered in sufficiently large doses by specific routes of administration. In place of the word poison, we will use the words "drug(s)" or "chemical(s)."

1.1.3 DRUG

The word "drug" derived from the Old French *drogue* by way of the Middle Dutch *drogue vate,* which referred to the dried goods contained in vats generally, is taken to mean a chemical that is used for a **beneficial medical purpose**.

Code of Federal Regulations (21CFR210.3, 2015) makes the following definitions under Rules for the Food and Drug Administration (with emphasis added):

- "Drug product means a finished dosage form, for example, tablet, capsule, solution, etc., that contains an *active drug ingredient* generally, but not necessarily, in association with inactive ingredients. The term also includes a finished dosage form that does not contain an active ingredient but is intended to be used as a placebo."
- Active ingredient means *any component that is intended* to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or *to affect the structure or any function of the body of man* or other animals. The term includes those components that may undergo chemical change in the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect.
- Inactive ingredient means any component other than an *active ingredient.*

Based on these definitions, we will attempt to adhere to the use of the word(s) "drug(s)" to refer to substances that are intended to furnish pharmacological activity or to affect the structure or any function of the body of man or other animals and are used intentionally or unintentionally for appropriate or inappropriate purposes. We will use the word(s) "chemical(s)" for those substances, e.g., volatile organic compounds, pesticide, carbon monoxide, that are not intended either for medical purposes or to affect the structure or any function of the body of man or other animals, but that are intentionally or unintentionally used or misused for the effects that they produce.

1.1.4 FORENSIC TOXICOLOGY

Forensic toxicology "… has no future as it is now organized and will not have until an adequate definition of forensic toxicology is reached" (Kemp, 1974). This statement demonstrates the confusion among forensic toxicologists that existed in the not-too-distant past as to a definition of their profession. Initially, forensic toxicology was referred to as "postmortem chemistry" and forensic toxicologists were referred to as "coroner's chemists" as the roles and functions that fell within the purview of the science and its practitioners were the detection and/or quantitation of drugs present in postmortem samples and the interpretation of the results obtained. Under these circumstances, forensic toxicology could be defined as the science concerned with determining whether the death of an individual was caused by, or related to, the use of a drug. This "classical" definition is consistent with the role of forensic toxicologists in a coroner's or medical examiner's office in which they are part of the team that investigates the possible role of drugs in fatalities. As a result of the additional demands placed on forensic toxicologists by society, forensic toxicology has become a much broader discipline in that it presently encompasses additional aspects of toxicology, principally as they relate to the living.

Currently, there are considered to be three different types of forensic toxicology: postmortem toxicology, human-performance testing, and forensic urine drug testing. These have been defined as follows (SOFT/AAFS), 2006).

- "Post-Mortem Forensic Toxicology, which determines the absence or presence of drugs and their metabolites, chemicals such as ethanol and other volatile substances, carbon monoxide and other gases, metals, and other toxic chemicals in human fluids and tissues, and evaluates their role as a determinant or contributory factor in the cause and manner of death.
- Human-Performance Forensic Toxicology, which determines the absence or presence of ethanol and other drugs and chemicals in blood, breath or other appropriate specimen(s), and evaluates their role in modifying human performance or behavior.
- Forensic Urine Drug Testing,[1] which determines the absence or presence of drugs and their metabolites in urine to demonstrate prior use or abuse."

The classical definition of forensic toxicology describes the discipline as retrospective, in that its aim is to determine whether there is a correlation between an event of interest and any drugs detected after the occurrence of such an event. The more recent description of the field includes a prospective aspect of forensic toxicology, such as preemployment drug screening, in which an attempt is made to identify the potential hazards of drug use by a person before the drug use causes any adverse effects.

1.2 LANDMARKS IN FORENSIC TOXICOLOGY

1.2.1 EARLY ACTIVITY IN TOXICOLOGY

It seems reasonable to assume that throughout history humans have been concerned with the adverse effects produced by the numerous substances they have

[1]This category should be expanded to include the detection of drugs in hair and oral fluid as these samples are being used for the same purposes as urine drug testing.

encountered in their environment. The written expression of this concern dates back at least as far as the *Ebers Papyrus* (Sigerist, 1951, p. 311), which is a record of medical knowledge and practices in Egypt from approximately 1550 BC and which describes naturally occurring toxic substances such as hemlock, opium, and lead as well as their antidotes—including those that are not only ineffective and/or harmful, but also repugnant. In the fourth-century BC, several dangerous plants were described in the *De Historia Plantarum* written by the Greek botanist and philosopher Theophrastus (Gallo, 2001). In the first-century AD, the Greek physician Pedanius Dioscorides, who served with the Roman army of the emperor Nero, wrote the *Materia Medica*—Dioscorides is credited with the first classification of poisons into separate classes such as plants, animals, and minerals (Haas, 1996).

The *Hsi Yuan Lu*, translated variously as or "Translations to Coroners" or "The Washing Away of Wrongs" (Kiel, 1970; McKnight, 1981), a multivolume series of books of legal medicine from the thirteenth-century AD China, is thought to be the oldest extant book on forensic medicine (Agren, 1984). This work includes a list of the duties and responsibilities of the district magistrate, the chief governing official for a governmental administrative area. Among the several duties of the magistrate was the investigation of suspected homicides, including poisonings. In this duty, the magistrate was aided by his assistant, the coroner, in performing the investigation and postmortem examinations as directed by the *Hsi Yuan Lu*. Although the *Hsi Yuan Lu* predates by centuries the scientific era of toxicology, it contains several methods that exemplify early attempts at "scientific" toxicology. One method called for the insertion of a silver needle into the mouth or body cavity of the deceased (McKnight, 1981, p. 135); blackening of the needle was taken as a sign of a poisoning. Although there is a scientific explanation for the blackening of the needle since silver can react with sulfur-containing compounds to form black precipitates, this method is obviously inadequate and falls short of modern requirements of proof, since most likely the black precipitates produced would be due to the reaction of the silver with hydrogen sulfide, a product of putrefaction and not the detection of a poison (Kiel, 1970). A second procedure relied on biological rather than chemical detection (Giles, 1924). Boiled rice was placed in the mouth of the deceased where it was kept for several hours after which it was fed to a chicken. The effect, if any, on the chicken was noted. Although this procedure has not caught on with forensic toxicologists, the use of animals in forensic toxicology persisted for many years (Of Interest 1.1). As primitive as they were, the developers of these early attempts at "scientific toxicology" should be applauded for their ingenious application of observations in an attempt to solve theretofore insoluble problems.

In the sixteenth century, Philippus Theophrastus Aureolus Bombastus von Hohenheim, more commonly and better known as Paracelsus, formulated his famous maxim: "In all things there is a poison, and there is nothing without a poison. It depends only upon the dose whether a poison is poison or not" (Ball, 2006, p. 229). Paracelsus, an alchemist, theologian, physician, and "protoscientist," rejected

OF INTEREST 1.1 THE ANALYTICAL FROG

Although the development of the Marsh test and subsequent other tests for the detection of arsenic in biological samples had been developed prior to the middle of the nineteenth century, adequate chemical methods were not available for the detection of many homicidal substances. For this reason, biological tests, somewhat more sophisticated than those described in the *Hsi Yuan Lu*, which were conducted using animals for the detection of these substances, persisted well into the late nineteenth century.

Reese, a leading toxicologist of the time, suggested a number of animals that would be suitable for use in toxicological testing—cats, rabbits, guinea pigs, or mice were recommended, but not birds which were deemed to be unsatisfactory for this purpose (Reese, 1889). One such method, for the detection of strychnine, a convulsive drug, reported by Reese relied on the use of frogs, which were reported to be sensitive to the effects of strychnine. This method was recommended since other substances, such as morphine, were known to interfere with other, nonanimal-based tests for the detection of strychnine in biological samples. The method described by Reese consisted of the subcutaneous injection into a frog of an extract of stomach and stomach contents obtained from the body of a person suspected of having been poisoned by strychnine. A positive result for strychnine by this method was the production of spasms in the animal. Since this test was also nonspecific for strychnine, it was suggested that it should be used in conjunction with smell, taste (the early forensic toxicologists were fearless), and color tests of the extract prepared from the stomach and stomach contents.

the works of Galen[2] that had prevailed for centuries and instead promulgated, among several other and generally less accurate theories, a far from modern chemical theory of diseases in his *Opus paramirum* (Ball, 2006, p. 260) in which he considered the cause of disease to be a bodily imbalance of three substances—salt, mercury, and sulfur. During his life, Paracelsus who was at times "looked upon as a magician and quack and sometimes as a physician of genius" by his contemporaries (Sigerist, 1951, pp. 12−14), was drunk for a good portion of his life, was castigated as a disciple of the devil (Ball, 2006), and failed to cooperate with his contemporaries—many of whom he treated with outright contempt and scorn (Davis, 1993). Nonetheless, regardless of his personal and professional shortcomings, this antisocial polymath is remembered today as perhaps the first to recognize the significance of dose and of the harmful potential of all substances. Considering the scientifically barren times in which he lived, we must excuse his failure to recognize that other factors, such as the route of administration, gender, age, and genetics may account for the differentiation among beneficial, innocuous, and harmful effects.

Although alchemists and protoscientists continued their attempts throughout subsequent centuries to understand the effects of chemicals on the human body, it was not until the development of the basic disciplines of chemistry and biology that modern, or truly scientific, toxicology developed. In the early nineteenth century, Mathieu Joseph Bonaventure Orfila (Figure 1.1), generally referred to as "The Father

[2]Galen, who lived in the second-century AD, is considered to be the greatest physician and medical researcher of antiquity. Many of his theories of physiology, anatomy, and pathology, although containing several errors and mistaken concepts, persisted in to the sixteenth and seventeenth centuries.

FIGURE 1.1

Mathieu Joseph Bonaventure Orfila.

of Toxicology," was at the forefront of the establishment of the scientific foundation of modern toxicology.[3] He studied the biological and chemical characteristics of several toxic substances and developed and applied methods of chemical analysis of postmortem materials to determine whether death was caused by a toxic substance. One of his most important findings was that drugs were absorbed into the blood and distributed to the tissues of the body and therefore could be detected in tissues other than those of the gastrointestinal tract (Coley, 1991). In 1813–1814, Orfila published his classic two-volume reference, *Traité de Toxicologie: Traité des poisons tires des regnes* minéral, végétal at animal ou toxicologie générale considerèe sous les rapports de la physiologie, de la pathologie et *la mèdicine legale*, which is considered to be the first book of modern toxicology (Borzelleca, 2001). In this work, he classified substances into six categories: corrosives, astringents, acrids, stupefying and narcotics, narcotic-acrids, and septics or putrefiants. This presentation of toxicological principles and concepts was an immediate scientific sensation and translations soon appeared in several countries including an 1817 abridged translation, *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*, in the United States by Joseph Nancrede.

[3]Orfila was also active in other areas of forensic science. For example, he published papers on the chemical identification of bloodstains following their aqueous extraction (Gaensslen, 1983, p. 74).

The Industrial Revolution and the continuing development of chemistry and biology in the nineteenth century and the subsequent development of analytical chemistry, biochemistry, physiology, pharmacology, anatomy, pathology, and statistics fostered the inception and growth of diverse toxicological disciplines including analytical toxicology, clinical toxicology, environmental toxicology, veterinary toxicology, genetic toxicology, regulatory toxicology, and forensic toxicology. The interdisciplinary nature of toxicology is demonstrated by the number of scientific disciplines to which it has been applied. It is unlikely that toxicologists will have expertise in all of the foundational disciplines of toxicology, but they must have at least a working knowledge of many and an extensive knowledge of one or more of these disciplines depending upon their areas of specialization.

Orfila and many of the first scientists to refer to themselves as toxicologists were concerned with the detection of homicidal poisonings. These early forensic toxicologists, who generally came from careers in medicine, were crucial to the development and establishment of the three basic roles of their maturing science: analysis, interpretation, and reporting. These forbearers of the discipline developed chemical methods of analysis that could be applied to postmortem samples, applied their knowledge of the basic sciences to the interpretation of the analytical results, and presented their findings in a manner acceptable to and understood by judges and juries. In short, they identified and established the roles and functions of present-day forensic toxicologists.

Presented below is a discussion of a selected group of events and scientists, which when taken together serve to illustrate the early development of forensic toxicology.

1.2.2 ARSENIC

The late eighteenth and early nineteenth centuries saw the continuing development of the biomedical sciences including the "new" science of toxicology, which was heavily dependent upon advances in chemistry and physiology. Prior to the development of chemistry, the absence of reliable chemical and toxicological methods of analysis made the detection of drugs and chemicals, especially in biological samples, difficult and generally unreliable. As a result, suicidal, homicidal accidental poisonings, by means of naturally occurring materials such as minerals and plant-derived substances, were widespread.

Arsenic is one of the naturally occurring chemicals that has been used widely throughout history as a favored instrument of suicide and homicide, perhaps even having had an influence on history.[4] In addition to its homicidal use, it was also

[4]Livia, the wife of the Roman emperor Augustus, was rumored to have been one of the most notorious arsenic murderers. She was said to have been responsible for several murders committed with arsenic, including that of Augustus, so that her son could ascend to the throne. Her exploits served as the focus in the historical fiction, *I, Claudius*, by Robert Graves.

widely available during the nineteenth century as a means of rodent control, as the active agent in sheep dip used to prevent infestations of farm animals, in foods, household remedies, and in the form of copper arsenite ($CuHAso_3$), it was the pigment in Scheele's Green, popularly used for imparting a green color to several products including in paints and wallpaper. Because of its pervasiveness in society, arsenic played a central role in the development of legal medicine and because of this was instrumental in the development of forensic toxicology in the nineteenth century.

The popularity of arsenic, usually in the form of the trivalent As_2O_3 or "white arsenic" as a homicidal agent, is illustrated by reports that it was the leading cause of known homicidal poisonings in the early nineteenth century (Watson, 2006a) and that it was the cause of 185 of the 541 recorded cases of fatal poisonings in England in 1837—1838 (Coley, 1991). There were several reasons for the popularity of As_2O_3 as a homicidal agent: it was inexpensive, readily available, had a sugar-like appearance, and had little smell or taste, which enabled the poisoner to mask easily its presence in food or drink. Additionally, the signs and symptoms (Ellenhorn, 1997, p. 1540) produced by arsenic ingestion, including severe abdominal pain, diarrhea and vomiting, and inflammation of the gastrointestinal tract, were similar to other causes such as cholera, the occurrence of which into the nineteenth century was not rare. For these reasons, and, probably most importantly, because of the lack of a reliable method for the detection of arsenic in human remains, the use of arsenic as a homicidal agent flourished in the early nineteenth century.

Physicians recognized that in order to establish that arsenic poisoning was the cause of death in suspected homicides, a reliable method was required by which arsenic could be detected in human samples. This need to identify homicidal poisonings by the reliable detection of arsenic, and by extension of other agents, was an important stimulus to, and paralleled the development of forensic toxicology.

The identification of arsenic in the eighteenth and early nineteenth centuries commonly relied on methods that are now considered primitive, such as the production of a garlic-like (alliaceous) odor when arsenic-containing substances were heated; reduction by which arsenic present in samples was reduced to its elemental form by heating; and prominently, "the liquid tests" that consisted of the use of various reagents that would produce characteristically colored precipitates consistent with the presence of arsenic (Of Interest 1.2).

The liquid tests included the reaction of samples with reagents such as ammoniacal sulfate of copper (copper sulfate in ammonia), ammoniacal nitrate of silver (silver nitrate in ammonia), lime water, or sulfuretted hydrogen (hydrogen sulfate) (Burney, 2002), which were expected to react in the presence of arsenic to produce colored precipitates. These tests were not easily adaptable to the detection of arsenic in biological samples since they were difficult to perform, had relatively high detection limits, were subject to errors of specificity, and were not easily adaptable to colored biological samples (Burney, 2002). Importantly, the end points of the analyses, the formation of precipitates of specific colors, required extensive training to recognize, were by their nature subjective due to interpersonal variation in color

OF INTEREST 1.2 *ON THE ROAD TO MARSH* (CAMPBELL, 1965; CAUDILL, 2009; FARRELL, 1994; GOLDSMITH, 1997)

The need for a reliable method for the detection of arsenic produced a number of methods, many of which were in common use prior to Marsh's landmark discovery; all were supplanted by the Marsh test.

Carl Wilhelm Scheele, 1775: Developed a method for the production of arsine (AsH_3) in nonbiological samples.

$$As_2O_3 + 6Zn + 12HNO_3 \rightarrow 2AsH_3 + 6Zn\ (NO_3)_2 + 3H_2O$$

Samuel Hahnemann, 1785: Developed a test in which the passage of sulfureted hydrogen gas through an acidified arsenic solution to produce a bright yellow precipitate of arsenius sulfide.

$$H_2S + HCl \rightarrow As_2S_3$$

Johann Daniel Metzger, 1787: Determined that heating arsenic trioxide with charcoal would reduce it to its elemental form, a method known as the reduction test.

$$2As_2O_3 + 3C \rightarrow 3CO_2 + 4As$$

Benjamin Rush, 1805: Identified the reaction of arsenites and arsenates with alkaline copper sulfate to produce a green precipitate.

$$3Cu_{2+} + 2(AsO_4)^{-3} \rightarrow Cu_3(AsO_4)_2\ (s)$$

Valentine Rose, 1806: Applied the Metzger's method to the detection of arsenic in gastric tissue.

Joseph Hume, 1809: Described the reaction between silver nitrate with arsenites to form a yellow precipitate.

$$3AgNO_3 + AsO_3{}^{-3} \rightarrow Ag_3AsO_3$$

recognition, and were described in specific terms that had unclear meanings, e.g., "the bloom of an Orleans peach," "lively" grass green, and "brilliant" lemon yellow (Burney, 2006).

Although these methods of detection were nonspecific, subject to errors of interpretation and generally not applicable to biological samples, they were accepted as scientific evidence in trials of the time (Of Interest 1.3).

The problems in the application of the "liquid tests" to complex samples served to spur interest in the development of analytical and forensic toxicology. In 1813, Orfila attempted to demonstrate to his students in Paris that the liquid tests could be used to detect arsenic in complex samples (Nieto-Galan and Bertomeu-Sanchez, 2006). To his dismay, the precipitates that formed when the reagents were added to a sample of coffee to which he had added arsenic were not of the anticipated colors. As a result of these unexpected results, Orfila is said to have exclaimed—"Toxicology does not exist." His extensive ground-breaking scientific efforts following this episode were instrumental in the writing of his classic work, *Traité de Toxicologie*. Publication of *Traité de Toxicologie*. This book and Orfila's research, which included the development of analytical methods for the detection of poisons and the demonstration that chemicals were absorbed into the general circulation, were momentous events in the development of toxicology as a scientific discipline and led to Orfila being celebrated deservedly today as the "Father of Toxicology."

OF INTEREST 1.3 WHAT A "GRUEL" DEED (ANONYMOUS, 1752; EMSLEY, 2005, PP. 145–147)

> *I forgive thee my Dear and I hope God will forgive thee; but thee shouldst have considered better, before thee attemptist any Thing against thy Father; thee shouldst have considered I was thy own Father.*

This statement was made shortly before his death by Francis Blandy, who was convinced that his sickness had been caused by his daughter Mary. Mary Blandy, a 26-year-old "spinster" living in Henley-on-Thames fell in love with Lieutenant William Henry Cranstoun, a married man who hid his marital status from Mary. However, Cranstoun did not hide his desire to marry her, in spite of the objections of her father. Cranstoun's ardor no doubt was spurred on by the 10,000 pound dowry that Mary's future husband would acquire. Cranstoun convinced Mary that the "powders to clean Scotch pebbles" that he gave her, if administered to her father would change her father's resistance to their marriage. Mary, apparently extremely gullible, believed him and periodically added the powder to her father's food over a period of months, until a final dose of the powder added to his gruel in August of 1751 proved fatal. Mary was brought to trial in February of 1752 for the fatal poisoning of her father with arsenic trioxide.

Dr Anthony Aldington, who had cared for Mr Blandy, provided medical and scientific testimony for the prosecution. His medical opinions were based both on the classic signs and symptoms of arsenic poisoning—severe pain of the gastrointestinal tract accompanied with severe vomiting and diarrhea—that Mr Blandy exhibited after eating the gruel as well as on postmortem findings that were consistent with arsenic poisoning. Aldington's identification of arsenic was based on the detection of "… the Stench of Garlick" upon heating of samples and the results of several of the chemical color tests commonly used for the identification of arsenic. He summarized his results of these tests by testifying that a known sample of arsenic and the powder found in Mr Blandy's gruel.

> *… corresponded so nicely in each Trial that I declare I never saw any two Things in Nature more alike than the Decoction made with the Powder found in Mr. Blandy's Gruel and that made with white Arsenic."*

Mary Blandy was convicted and subsequently hanged on April 6, 1752.

Additional criticisms of the liquid test were levied by Sir Robert Christison (Figure 1.2), the preeminent forensic toxicologist of the nineteenth century in Great Britain:

> *If what has been said of the modifications which the liquid tests for arsenic undergo in their action when they are applied to vegetable and animal fluids be reconsidered it will at once be seen that they are quite useless in relation to such fluids. If the solution indeed contains a large proportion of arsenic and is not deeply coloured all the three will act in the usual manner. But in actual practice the solutions are always diluted and in them the liquid tests with the exception of sulphuretted hydrogen gas either do not act at all or throw down precipitates so materially altered in tint from those which alone are characteristic of their action that their employment would lead to frequent mistakes.*
>
> **Christison (1829)**

FIGURE 1.2

Robert Christison.

Christison's characterization of the problems of the liquid tests was accurate and carried great weight since Robert Christison was the preeminent toxicologist in Great Britain in the first half of the nineteenth century. His text, *A Treatise on Poisons in Relation to Medical Jurisprudence, Physiology and the Practice of Physic*, which was published in 1829 when he was professor of medical jurisprudence and police at the University of Edinburgh in Scotland, was the first work devoted to forensic toxicology in Great Britain (Anonymous, 1830) and the first book on toxicology written in English and published in the 19th century (Christison, 1829, p. i). This publication, his development of analytical methods, his success as an expert witness in forensic toxicology, and his position as medical adviser to the Crown in Scotland for 37 years (Coley, 1991), brought him such acceptance and fame that he felt "… his reputation in Scottish courts became so overpowering that his evidence was rarely questioned" (Crowther, 2006).

The problems of arsenic detection in human remains raised by Orfila, Christison, and others was successfully addressed first by James Marsh, a low-salaried chemist employed by the English government, whose work in this field was stimulated by the 1832 trial of John Bodle who had been charged with the murder of his tyrannical grandfather (Thorwald, 1964). Marsh had participated in this case as an expert for the prosecution and had conducted the prevailing standard color tests for the detection of arsenic. He reported the presence of arsenic in the coffee prepared by the defendant for his grandfather and he was confident of the defendant's guilt.

However, Bodle was found innocent. Marsh was convinced that his inability to present demonstrable evidence to the jury was instrumental in the acquittal.[5] As a result of his failure to convince the jury of his analytical findings in this case, Marsh worked to develop a method of analysis for the detection of arsenic in human tissues that would solve the courtroom and scientific problems associated with the existing methods. Based on the prior work of Carl Wilhelm Scheele[6] in 1775 and others (Watson, 2006a), Marsh developed a method, which now bears his name, that could be employed for the detection of arsenic in biological samples and would produce demonstrable positive results that a jury could see (Marsh, 1836). The basis of the Marsh test is the reaction of arsenic-containing samples including biological fluids or tissues with hydrogen gas generated by the reaction of zinc with an acid, such as sulfuric acid. When heated, arsine gas (As_2H_3)—the product of this reaction—is reduced to metallic arsenic that may be collected on a solid surface such as a glass or porcelain plate. The presence of the shiny deposit, known as an arsenic mirror, is a positive result. In the paper reporting the development of his method, Marsh stated that

> *Notwithstanding the improved methods that have of late been invented of detecting the presence of small quantities of arsenic in the food, in the contents of the stomach, and mixed with various other animal and vegetable matters[7] a process was still wanting for separating it expeditiously and commodiously, and presenting it in a pure unequivocal form for examination by the appropriate tests.*

The Marsh test was an analytical sensation because it presented forensic toxicologists with a method for the detection of arsenic in biological samples. Although the test was not specific for arsenic, it could be used for the detection of very small amounts of arsenic, was reliable in the hands of an experienced chemist, and produced demonstrable results that could be shown easily and explained to a lay jury comprised of individuals unfamiliar with analytical assays. However, in spite of its analytical merits, the Marsh test initially was met with mixed reviews. Alfred Swaine Taylor (Figure 1.3) (Coley, 1991; Rosenfeld, 1985), who had been appointed lecturer in medical jurisprudence at Guy's Hospital in London in 1831 and subsequently developed a widespread reputation and fame as a forensic toxicologist due to his textbooks in medical jurisprudence as well as his effectiveness as an expert witness, was an early advocate of the Marsh test, although in certain cases he deemed it to be unnecessary and relied on more traditional methods of detection.

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[5]The ability to convince jurors of the validity of scientific evidence is perhaps the most important role of the expert at trial, but it is also one of the most difficult.

[6]Scheele has been credited with the discovery of oxygen years prior to the claims of Priestley, who is generally credited with the discovery, or Lavoisier, who claimed the priority of discovery (Severinghaus, 2003).

[7]Unfortunately, neither the work of Sheele nor any of the others who developed the methods to which he referred and who laid the foundation for his breakthrough was mentioned by Marsh in the paper describing his method.

FIGURE 1.3

Alfred Swaine Taylor.

Less enthusiasm for the Marsh test was expressed by Fresenius, the renowned German chemist who created the first journal dedicated exclusively to analytical chemistry. Fresenius opined that the Marsh test was not suitable for the detection of arsenic in organic matter and that there was a possibility that zinc and sulfuric acid used in the test could be contaminated with arsenic (Coley, 1991). However, because Marsh had been aware of the "ambiguity" (false-positive results) that might result if his reagents or apparatus were contaminated with arsenic, he had recommended that the procedure should be performed in the absence of a sample to ensure that any arsenic that was detected did not originate from either of those sources. He described the analysis of a blank (although he did not use that term) consisting of the zinc and sulfuric acid reagents in the absence of a sample as follows:

> *It is, therefore, necessary for the operator to be certain of the purity of the zinc which he employs, and this is easily done by putting a bit of it into the apparatus, with only some dilute sulfuric acid; the gas thus obtained is to be set fire as it issues for the jet; and if no metallic film is deposited on the bit of that glass, and no white sublimate within the open tube, the zinc may be regarded as in a fit state for use.*
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> **Marsh (1836)**

Marsh's method not only greatly improved existing methods, but it also stimulated the development of other methods for arsenic detection by Berzelius and Reinsch, who developed a method by which arsenic and other metals were detected by their plating onto a copper coil in a boiling HCL solution (Reinsch, 1842). Additionally, Gutzeit developed a semiquantitative method for arsenic detection in which arsine gas is reacted with nitric acid to produce a precipitate, which, with numerous modifications, was used into the twentieth century.

The Marsh test had ushered in the era of scientific analytical toxicology and with it the modern age of forensic toxicology.

1.2.3 THE LAFARGE AFFAIR (SAUNDERS, 1952; THORWALD, 1964)

The Marsh test played a prominent role in a case of homicidal poisoning that came to be known as the LaFarge affair. This case provoked the same type of widespread public attention in the nineteenth century as the O.J. Simpson case did in the twentieth century.

The principal characters in the LaFarge affair were Marie Cappell and her husband, Charles LaFarge. Before they were married, Charles LaFarge had represented himself to Marie as the owner of a thriving foundry and a fine country estate, neither of which was true, and which caused a great distress to Marie when she first saw the "estate" after her marriage to this man who she hardly knew. In December 1839, shortly after their marriage, while Monsieur LaFarge was in Paris on a business trip, he received a cake prepared for him by his wife. Charles became ill after eating the cake and returned home where he was cared for by Marie. In spite of or, as later was charged, because of Marie's care, Charles died on January 13, 1840. Some of the servants on the LaFarge estate were suspicious of Madame LaFarge's behavior (she would not allow anyone other than herself to care for her husband) and suspected foul play. As a result of their investigation, which, among other findings, revealed that Madame LaFarge had purchased arsenic in December 1839—prior to Monsieur LaFarge's trip to Paris, the authorities concluded that Madame LaFarge had poisoned her husband and she was charged with homicide.

In addition to the nonscientific evidence that they uncovered, the authorities made several attempts to determine whether the remains of Charles LaFarge contained arsenic. A panel of "experts" comprised of physicians from Brives was called upon to conduct analyses of the exhumed remains of Charles. They reported that they had detected arsenic in LaFarge's stomach and stomach contents. However, Orfila, who was consulted by the defense, concluded that these physicians, who were unaware of the Marsh test, had used an outdated and nonspecific method of detection and their results were therefore not reliable. The court then appointed a second panel of "experts" consisting of two apothecaries and a chemist from Limoges. Responding to the criticism of the results produced by the physicians from Brives, they applied the Marsh test, a method they had never used before; they reported that they did not detect arsenic in LaFarge's stomach or stomach contents. In order to resolve the several discrepancies among the analytical results, the court then ordered a "tie-breaker" in which the "experts" form Brives and Limoges would work together to analyze samples from LaFarge's exhumed body to determine whether arsenic was detectable in any of the organs. The combined experts reported that arsenic was not detected in the organs obtained from the exhumed body. However, arsenic was detected in eggnog prepared for Charles by Marie and also in Marie's malachite box, which contained a white powder she had been seen putting in the eggnog.

In the midst of this scientific chaos, Orfila was called upon to examine LaFarge's remains. Employing what was then the state-of-the-art Marsh method for his determination of arsenic, Orfila analyzed the samples obtained from LaFarge's body and testified that he had detected arsenic in them.[8] Based on Orfila's scientific testimony and the investigative findings, Madame LaFarge was convicted and sentenced to life in prison, although her sentence was commuted after she had served a few years. Marie LaFarge's case was a *cause célèbre* and generated extensive scientific and popular tumult since she had many supporters who defended her innocence. She even wrote a memoir that was a popular success.

Orfila's work in the LaFarge case was received enthusiastically by many who welcomed it as the dawn of modern toxicology, which held the promise of detecting poisons as widely used as arsenic in the tissues of a victim by means of state-of-the-art chemical methods. Orfila's role in this case contributed to his eminence as "… one of the first international stars of science" (Crowther, 2006). However, the analytical results and the verdict were also greeted with controversy by those who argued that the Marsh test was subject to numerous errors of procedure and interpretation (Of Interest 1.4). Among the criticisms of the results obtained by Orfila by means of the Marsh test were (1) that the results did not agree with those produced by original experts and (2) the method was so sensitive that contamination of the postmortem samples by arsenic in the reagents or in the cemetery soil could have produced false-positive results. However, Orfila had conducted analyses and obtained data that anticipated and blunted these, as well as other criticisms. He explained that the inconsistency between his results and those of the local "experts" was due to their lack of expertise in the performance of the test, e.g., they used samples that were too small, they used a flame that was too large, and they did not wait long enough for the formation of the arsenic deposit. The second criticism was discounted since he had demonstrated that neither the reagents he used nor the soil from the cemetery contained arsenic, as determined by the Marsh test (Of Interest 1.5). In addition, Orfila explained that the arsenic he had detected in the samples taken from LaFarge's body was present in a quantity that was much greater than the amount of arsenic found naturally in the human body.

The LaFarge affair demonstrated that newer methods of chemical analysis employed in the detection of chemicals from postmortem samples were reliable only if precautions were taken to avoid contamination of samples and if they

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[8]Orfila was eminently qualified for these analyses since he was the first to extract arsenic from nongastrointestinal organs (Eckert, 1980).

OF INTEREST 1.4 HE SHOULD HAVE TAKEN THE TRAIN (WEINER, 1959)

One of Orfila's leading critics was François Vincent Raspail, a distinguished scientist in his own right who has been called the "founder of microchemistry" and who formulated an early version of the cell theory.

Orfila and Raspail disagreed not only about scientific matters, but they also held differing political views, which may have exacerbated their scientific disagreements—Raspail was an antimonarchist republican who was jailed and exiled for his political views, whereas Orfila supported the monarchy. One of the longest boulevards in Paris is named for Raspail.

Raspail was to testify for the defense in the LaFarge case, but did not arrive at the court in time to do so because he fell from his horse in his haste to reach the court in Tulle (Thomas, 1974).

OF INTEREST 1.5 THE SOIL DID IT

Although the criticism in the LaFarge affair that arsenic in the soil had contaminated the remains of Charles LaFarge was answered by Orfila, the "soil did it" defense persisted into the twentieth century in the case of Marie Besnard (Thorwald, 1964) who was accused of the fatal arsenic poisoning of her husband and several relatives and neighbors. The exhumed bodies of several of her alleged victims were found to contain elevated concentrations of arsenic. After 3 trials, over a period of 9 years, Marie was acquitted of all charges, in part as a result of the defense position that the presence of arsenic in the exhumed bodies of the alleged victims may have resulted from the action of soil microbes that caused the diffusion of arsenic from the soil into the buried bodies.

were performed by scientists who were well trained, experienced, and expert in their use. These caveats remain to this day.

1.2.4 THE BOCARME CASE (ANONYMOUS, 1882; THORWALD, 1964; WENNING, 2009; WHARTON AND STILLÉ, 1855)

A second "crime of the century," the Bocarme case, was significant not only for its sensationalism, but also for its impact on the development of analytical and forensic toxicology. In 1843, Alfred Juliet Gabriel Hippolyte Visart, the Count de Bocarmé, married Lydia Fougnies, the daughter of a prosperous grocer, in the anticipation that financial gifts from her father would enable him to maintain his lifestyle—one that included a large mansion staffed with many servants, elaborate parties, and hunting expeditions. The Count soon realized that his wife's yearly income from her father's estate coupled with his own income was insufficient for the maintenance of his preferred lifestyle and he generated huge debts of several thousand francs. Gustav Fougnies, the Countess' brother, who had inherited the major portion of their father's estate was unmarried and had been in poor health since the loss of his leg. Bocarmé became impatient waiting for Gustav to die a natural death and therefore planned to murder him since Lydia, her brother's only heir, would inherit his sizable estate. His plans had to be accelerated when Gustav surprisingly announced his plans to marry. Of course, Bocarmé, who wished to spend his anticipated largess, desired to commit the murder in a manner that could not be identified as a homicide. He determined that poisoning would be the best way of achieving his goal.

Using an assumed name, Bocarmé approached Professor Löppens, a chemist, in Ghent for information concerning the preparation of nicotine from tobacco leaves.[9] Löppens described him the method to be used, and Bocarmé had the equipment necessary for the procedure manufactured. His first attempts were not successful, but ultimately, after almost a year of effort, Bocarmé obtained a sample of nicotine that was lethal to the animals on which he had tested it. After Bocarmé had prepared two vials of nicotine, an amount he judged to be sufficient for his purpose, he and his wife invited his brother-in-law to dinner at which time they attacked him and attempted to pour the nicotine down his throat. The brother-in-law resisted (some people just will not cooperate) and in the ensuing struggle, nicotine was splashed on his clothing and body as well as the floor. However, a sufficient amount was forced into the Gustav's mouth and he died. The Countess told the servants that her brother had died of apoplexy. After Gustav's death, Lydia directed the servants to wash or burn her brother's clothing and crutches and to wash the floor with vinegar. Vinegar was forced into Gustav's mouth and his body was washed with vinegar. The servants thought that the events of that evening and the behavior of the Bocarmés were unusual and suspicious and, therefore, reported their concerns to the authorities who initiated an investigation. Due to the suspicious behavior of the Count and Countess, the presence of chemical burns on the side of Gustav's mouth and a human bite mark on his hand, the authorities suspected that the cause of death was not apoplexy. Therefore, they had the body examined by physicians who concluded that there was no sign of natural death and that poisoning was indicated.[10]

Jean Servais Stas, a 37-year old, brilliant chemist at the École Royale Militaire was asked to determine whether any poisons could be detected in the tissues of Gustav Fougnies. It was widely accepted at this time that "vegetable alkaloids," i.e., nitrogenous bases found in plants, could not be detected in human tissue because of the complexity of the tissue matrix with its many potentially interfering substances that made it difficult to purify the alkaloids sufficiently to apply available methods of detection. Even the great Orfila, whom Stas had assisted in Paris, was of this opinion and had stated only a few years earlier that there was no accepted method for the extraction of vegetable alkaloids, such as nicotine, from human remains, and that the detection of these materials from human remains might never be possible (Wenning, 2009)!

[9]Bocarmé developed his methods under the ruse that he was preparing a unique *eau-de-cologne* or pesticide (Wenning, 2009)!

[10]The physicians erroneously surmised that the chemical burns were due to sulfuric acid; it was later concluded by Stas that they were due to the vinegar used by the Bocarmés.

However, Stas developed a method, now known as liquid—liquid extraction, by which the nicotine was extractable from samples into organic solvents. The method involved the separation of the nicotine from "animal matter" through a series of extractions of an alkalinized aqueous portion of the sample with ether. The residue that remained after the evaporation of ether was tested not only with the standard tests of the day for the identification of pure nicotine, but also by the odor of nicotine and that of mouse urine, an odor associated with nicotine—a unique *eau-de-cologne* indeed (Wenning, 2009)! On the basis of his analysis, Stas concluded that the body of the brother-in-law contained nicotine.

Based on the evidence presented by Stas as well as additional evidence developed by the investigators, the Count de Bocarme was convicted and guillotined. However, the Countess de Bocarme who said that she knew of her husband's activities and goals, but did nothing to stop him because her husband had threatened her and she feared for her life, was acquitted. Lydia indeed led a charmed life; shortly after her acquittal she received a bequest of several hundred thousand francs from the estate of an Englishman whose prior proposal of marriage she had refused (Anonymous, 1885).

The method of Stas was modified in 1851 by Otto for the removal of fats. The so-called Stas—Otto liquid—liquid extraction, although modified several times in the ensuing years, remains the basis for the liquid—liquid and solid-phase exactions used in forensic toxicology laboratories to this day.

Apart from its significance in the development of analytical toxicology, it is also of interest to note that the method developed by Stas, which was largely responsible for the conviction of Count de Bocarme, had been developed specifically for this case and had not been evaluated previously by other forensic toxicologists prior to the time at which the results obtained from its use were presented and accepted as trial evidence. The use and acceptance of a novel, untested analytical method in a criminal investigation was also significant in a case—the murder trial of Carl Coppolino—that would occur almost 100 years later.

In the nineteenth century, the work of Orfila, Christison, and Marsh spearheaded the development of forensic toxicology. The authors of several texts of medical jurisprudence attempted to incorporate forensic toxicology as an integral component of medical education and practice. However, by the late nineteenth and early twentieth centuries, the complexity of forensic toxicology had become "… too delicate for the medical profession" (Crowther, 2006), and it was entrusted to those scientists whose training and education had prepared them for this specialized profession. Forensic toxicology had become a science unto itself.

1.3 FORENSIC TOXICOLOGY IN THE UNITED STATES

The development of forensic toxicology which was taking place in Europe in the nineteenth century was slow in crossing the Atlantic. The publication of books in the United States in the mid-eighteenth century on the topic of medical jurisprudence (Niyogi, 1980) such as Dean's *A Manual of Medical Jurisprudence* in 1845 and *A Treatise on Medical Jurisprudence* in 1855 coauthored by Wharton, an attorney, and Stillé, a physician, devoted significant space to forensic toxicology issues, including general concepts of forensic toxicology and the diagnosis of poisoning by elements, organic and mineral acids, and various natural products. However, the book considered to be the first American book devoted to toxicology, *Microchemistry of Poisons*, by T.G. Wormley, was not published until 1867—more than 50 years after Orfila's classic work (Borzelleca, 2001). Wormley's text included a thorough presentation of the chemistry and toxicology of a number of poisons, as well as an overview of detection methods including, as appropriate, drawings of the crystals produced by the reaction of various substances with specific reagents. The success of the first edition led to the publication of a second edition in 1885 that was praised as meriting "… a separate place in medical literature occupying the middle ground between legal medicine and medical chemistry. To each of these branches it is an invaluable, and, we may say indispensable adjunct" (Anonymous, 1885). Subsequently, in the early twentieth century, several texts and research papers dealing with the symptoms and detection of poisons were published.

The landmark event in the development of forensic toxicology in the United States was the establishment of a forensic toxicology laboratory in the New York City Medical Examiner's Office in 1918, which followed the establishment of a medical examiner's system in that city (Freimuth, 1983). Alexander Gettler, the first director of this laboratory, took on this duty in addition to his duties as a pathological chemist at Bellevue Hospital and an instructor at the Bellevue Medical School (Freireich, 1969). Gettler and his laboratory staff developed or adapted methods for the detection for a number of substances including ethanol, methanol, carbon monoxide, cyanide, and chloroform and provided interpretations of analytical results. The thoroughness of Gettler's work is exemplified by his report that he evaluated 58 methods for the identification of methanol in approximately 250 liquors and more than 700 samples of human organs (Gettler, 1920)! In later life, he recounted the circumstances of several cases in which the presence or absence of substances such as fluoride, chloroform, and carbon monoxide led to the resolution of the cases (Gallo, 2001; Gettler, 1956). Not only was Gettler's direct influence on analytical and forensic toxicology extensive, but his influence ultimately spread far beyond New York as several of the forensic toxicologists whom he had trained disseminated their knowledge and skills throughout the United States. These scientists and the locations of their own laboratories include Henry Freimuth in Maryland; Leo Goldbaum at The Armed Forces Institute of Pathology; C.J. Umberger in New York; Irving Sunshine in Ohio; and Sidney Kaye in Puerto Rico (Eckert, 1980). These pioneers who had been trained by Gettler in turn trained a new generation of scientists, many of whom are active practitioners, who further disseminated the knowledge and special skills of forensic toxicology. Forensic toxicology truly is a young and continually developing scientific discipline in the United States.