9TH EDITION

Casarett & Doull's TOXICOLOGY

THE BASIC SCIENCE OF POISONS

CURTIS D. KLAASSEN

Mc Graw Hill Education

"What is there that is not poison? All things are poison and nothing (is) without poison. Solely the dose determines that a thing is not a poison."

Paracelsus (1493–1541)

Notice

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Casarett and Doull's
TOXICOLOGY

The Basic Science of Poisons

Ninth Edition

Editor

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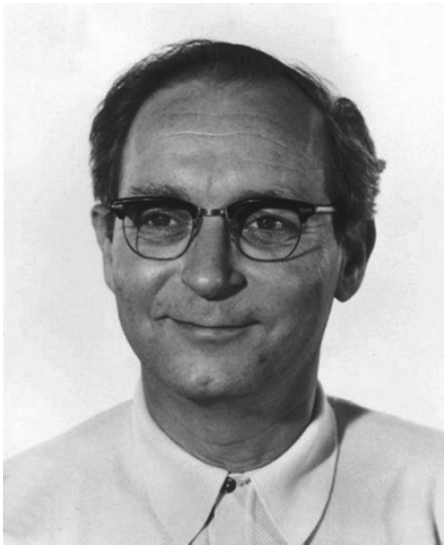
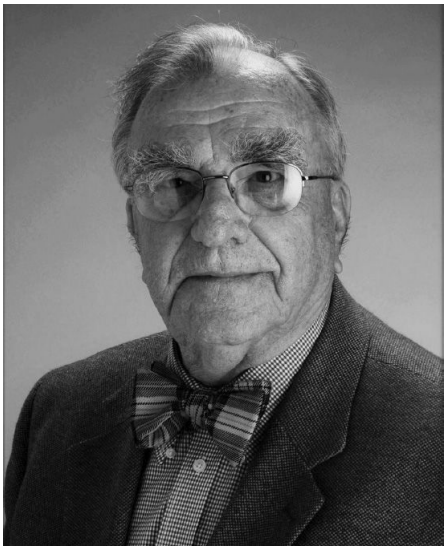
History and Dedication

Fifty years ago when I started lecturing graduate students there was no comprehensive toxicology textbook, and thus one often needed many hours in the library reading the literature to prepare for a lecture. Thus, I was thrilled when Lou Casarett and John Doull decided to edit a textbook in toxicology because it would enable me to give much better lectures with much less preparation time. The textbook provided a review of the literature on each topic in toxicology written by an expert in the area.



Louis James Casarett



John Doull

The origin of this textbook started at NIH Toxicology Study Sections meetings in the late 1960s and early 1970s. All members of the Study Sections agreed there was a growing need for a textbook in toxicology, in fact many members of those Study Sections became authors of various chapters in the book.

At the time, Lou Casarett was a professor at the University of Hawaii and John Doull was a professor at the University of Kansas. As a result, Lou spent time in Kansas City with John selecting authors of the book, whereas John and his family spent a summer in Hawaii in finalizing the organization of the book and writing chapters for

the first edition. Unfortunately, shortly thereafter and before the first edition was published, Lou died of brain cancer.

Klaassen, Amdur, Doull

The first edition was entitled *Toxicology: The Basic Science of Poisons* and was published in 1975. John Doull asked Mary Amdur, a friend of Lou Casarett, and myself, a younger toxicologist at the University of Kansas, to help him edit the second edition of the textbook. Mary suggested that the names of the two first editors be added to the title of the textbook, and thus the second and all subsequent editions have been entitled *Casarett and Doull's Toxicology: The Basic Science of Poisons.* The second, third, and fourth edition were edited by Doull, Amdur, and Klaassen. Mary Amdur died in 1998 and John Doull in 2017.

This ninth edition is dedicated not only to Lou Casarett, John Doull, and Mary Amdur, but all authors who have contributed to the nine editions of this book. These authors have summarized the knowledge in their area of expertise to help faculty prepare lectures as well as to help students learn the discipline. To emphasize the importance that previous authors have had on the education of toxicologists over the decades, their names are acknowledged in the chapter they previously authored.

Lou Cantilena, MD, PhD, author of the "Clinical Toxicology" chapter of this book and previous editions, was killed, along with his daughter, in an airplane accident in December 2017. Lou was piloting his daughter home for the Christmas holiday from Kansas City, where she was finishing her MD and PhD studies at the University of Kansas. Professionally, Dr. Cantilena will be remembered for his contributions to the Poison Control Centers and for treating poisoned patients, educating physicians for the military, doing clinical trials in order to discover more effective and less addicting treatments for pain, and consulting with the Food and Drug Administration on the management of drug-induced *torsades de pointes*. Lou's positive attitude, enthusiasm, smile, sincerity, and devotion to his family are hallmarks of his legacy.

Curtis D. Klaassen, PhD, DABT, ATS, FAASLD

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*Deceased

Preface

The ninth edition of *Casarett and Doull's Toxicology: The Basic Science of Poisons*, as in previous editions, is meant primarily as a text for, or an adjunct to, graduate courses in toxicology. Because the eight previous editions have been widely used in courses in environmental health and related areas, an attempt has been made to maintain those characteristics that will again provide information on the many facets of toxicology, especially the principles, concepts, and modes of thoughts that are the foundation of the discipline. Mechanisms of toxicity are emphasized. Research toxicologists will find this book an excellent reference source to find updated material in areas of their special or peripheral interests.

The overall framework of the ninth edition is similar to that of the previous editions. The seven units are General Principles of Toxicology (Unit I), Disposition of Toxicants (Unit II), Non-Organ-Directed Toxicity (Unit III), Target Organ Toxicity (Unit IV), Toxic Agents (Unit V), Environmental Toxicology (Unit VI), and Applications of Toxicology (Unit VII).

This edition reflects the progress made in toxicology during the last few years. The examples are the importance of apoptosis, autophagy, cytokines, growth factors, oncogenes, cell cycling, receptors, gene regulation, protective mechanisms, repair mechanisms, transcription factors, signaling pathways, transgenic mice, knock-out mice, humanized mice, polymorphisms, microarray technology, second-generation sequencing, genomics, proteomics, epigenetics, exposome, microbiota, read across, adverse outcome pathways, high-content screening, computational toxicology, innovative test methods, organ-on-a-chip, etc. in understanding the mechanisms of toxicity and the regulation of chemicals. This edition is markedly updated from the previous edition; over one-third of the chapters in this ninth edition are authored by scientists that have not been previously involved with the textbook. References in this edition include not only traditional journal and review articles, but Internet sites too.

Preface to the First Edition

This volume has been designed primarily as a textbook for, or adjunct to, courses in toxicology. However, it should also be of interest to those not directly involved in toxicologic education. For example, the research scientist in toxicology will find sections containing current reports on the status of circumscribed areas of special interest. Those concerned with community health, agriculture, food technology, pharmacy, veterinary medicine, and related disciplines will discover the contents to be most useful as a source of concepts and modes of thought that are applicable to other types of investigative and applied sciences. For those further removed from the field of toxicology or for those who have not entered a specific field of endeavor, this book attempts to present a selectively representative view of the many facets of the subject.

Toxicology: The Basic Science of Poisons has been organized to facilitate its use by these different types of users. The first section (Unit I) describes the elements of method and approach that identify toxicology. It includes those principles most frequently invoked in a full understanding of toxicologic events, such as dose–response, and is primarily mechanistically oriented. Mechanisms are also stressed in the subsequent sections of the book, particularly when these are well identified and extend across classic forms of chemicals and systems. However, the major focus in the second section (Unit II) is on the systemic site of action of toxins. The intent therein is to provide answers to two questions: What kinds of injury are produced in specific organs or systems by toxic agents? What are the agents that produce these effects? A more conventional approach to toxicology has been utilized in the third section (Unit III), in which the toxic agents are grouped by chemical or use characteristics. In the final section (Unit IV) an attempt has been made to illustrate the ramifications of toxicology into all areas of the health sciences and even beyond. This unit is intended to provide perspective for the nontoxicologist in the application of the results of toxicologic studies and a better understanding of the activities of those engaged in the various aspects of the discipline of toxicology.

It will be obvious to the reader that the contents of this book represent a compromise between the basic, fundamental, mechanistic approach to toxicology and the desire to give a view of the broad horizons presented by the subject. While it is certain that the editors' selectivity might have been more severe, it is equally certain that it could have been less so, and we hope that the balance struck will prove to be appropriate for both toxicologic training and the scientific interest of our colleague.

L.J.C.
J.D.

Although the philosophy and design of this book evolved over a long period of friendship and mutual respect between the editors, the effort needed to convert ideas into reality was undertaken primarily by Louis J. Casarett. Thus, his death at a time when completion of the manuscript was in sight was particularly tragic. With the help and encouragement of his wife, Margaret G. Casarett, and the other contributors, we have finished Lou's task. This volume is a fitting embodiment of Louis J. Casarett's dedication to toxicology and to toxicologic education.

J.D.

Dose and Dose-Rate matter

chapter 1 | The Evolving Journey of Toxicology: A Historical Glimpse

Philip Wexler and Antoinette N. Hayes

ABOUT TOXICOLOGY

Humans are smart but vulnerable. We need to be prepared for countless unforeseen events that could compromise our health and well-being. Toxicology arose as a way to understand, prevent, mitigate, and treat the potentially harmful consequences of many of the substances we are exposed to.

According to the Society of Toxicology (SOT) (http://www.toxicology.org/about/vp/vision.asp):

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.

The National Library of Medicine's (NLM) *Collection Development Manual* elaborates by noting:

Toxicology studies the agents responsible for adverse effects, the mechanisms involved, the damage that may ensue, testing methodologies to determine the extent

of damage, and ways to avoid or repair it. Toxicology is traditionally associated with chemical exposures, such as the effects of drugs, industrial chemicals, pesticides, food additives, household products, and personal care items. Toxinology, a sub discipline of toxicology, studies biological exposures, such as insect stings, poisonous mushrooms and plants, venomous snakes and aquatic life. The third category of toxicology is concerned with physical hazards, such as radiation and noise.

One of the key points to understand, as noted above, is that although toxicology in the popular mind is confined to chemicals and, probably, in practice most of the research and concern occur in this realm, other agents such as radiation and substances derived from biological organisms are equally relevant to the field.

The word *toxicology* is derived from the Latinized form of the Greek word *toxicon*, meaning "arrow poison." *Poison*, as a noun, dates back to the Old French *poison* or *puison*, meaning, originally, a drink, especially a medical drink, but later signifying more of a magical potion or poisonous drink. Another point of terminology concerns the commonly misused term *toxin*. Despite past and informal uses of the term, it formally should be used to refer to toxic substances produced biologically. Thus, technically, chemicals such as formaldehyde or asbestos, say, would *not* be considered toxins. There are any number of other terms which could be used to delineate the broader category of substances which are toxic, regardless of origin. Examples are *toxicant, toxic agent*, and *toxic substance. Xenobiotics* is a term referring to substances, whether toxic or not, foreign to a given organism.

Finally, in this brief lesson on toxicology nomenclature, one needs to clarify the use of the words *poisonous* and *venomous* when used as animal adjectives. Though often used interchangeably, they are, in fact, rather distinct. A venom requires a delivery mechanism. Thus, because a snake, for example, injects its venom (or toxin) into its victim, it is considered a *venomous* animal. Instead, a toxic mushroom must be ingested to make its effect felt. Thus, it should instead be deemed *poisonous*.

Toxicology is largely concerned with the interaction of toxicants and biological organisms. While toxicodynamics investigates the effect of the toxicant on the organism, toxicokinetics looks at how the organism affects the toxicant (e.g., absorption, biotransformation, distribution, and elimination). Mechanisms of toxicity at cellular and biochemical levels play a key role in determining why an agent has the effects it does. Toxic responses may be directed to particular organs or systems, for example, kidney, liver, and nervous system. Another way to consider effects is as clastogenic or mutagenic, resulting in carcinogenic or teratogenic effects. Often the focus of research is on a particular chemical or class of chemicals, such as pesticides, metals, or solvents. Environmental contamination and toxicology are tightly bound fields of study, and toxicology has much to contribute to an understanding of air, water, and soil pollution. Establishing the safety of drugs relies upon toxicology as does ensuring the safety of our water and food supply. Envenomations, whether by snakes, spiders, scorpions, aquatic life, or other creatures, as well as poisoning by plants and fungi are also within toxicology's scope.

Toxicology today is a highly interdisciplinary science that borrows from and intersects with other sciences such as chemistry, biology, pharmacology, medicine, physiology, biochemistry, molecular biology, pathology, and environmental science. Increasingly, it is also appropriating the tools of the computational sciences as one way to improve the precision of safety assessment, screen large numbers of chemicals efficiently, cut costs, and reduce animal use. Toxicology can be parsed into branches in a variety of ways. One such set of groupings follows:

Descriptive Toxicology: The emphasis is on the testing of toxicants, typically on animals. It focuses on the dose–response relationship and extrapolation to humans.

Mechanistic Toxicology: Looks at how the agent induces its biochemical or physiological effect on the organism, that is, modes of action. *Biochemical and Molecular Toxicology* is a synonym for this branch.

Clinical Toxicology: This branch's focus is on the effects of drugs and other chemicals on humans, particularly, but also on other animals. Its work is often involved with drug overdoses and other poisonings, and determining the substance involved and its amount in the body. Sometimes used synonymously with *Medical Toxicology* although technically, in terms of profession, a medical toxicologist tends to have an MD while a clinical toxicologist has a PharmD. A veterinarian who specializes in toxicology, typically, has a DVM.

Forensic Toxicology: Concerned with the cause of death from toxic agents, often in instances of drug abuse or misuse. With a focus on homicides and suicides, this branch of toxicology goes hand-in-hand with the work of the police and medical examiners.

Environmental Toxicology: Investigates the effects of toxicant exposures on the general environment and living organisms therein. Thus, pollution of air, water, and soil, and effects on plants and wildlife would fall within this branch. Ecotoxicology, a more specialized area, is devoted to the effects of toxic chemicals on populations,

communities, and terrestrial, freshwater, and marine ecosystems. Environmental toxicologists can further define their work in even more specialized terms, for example, aquatic toxicology.

Occupational Toxicology: Deals with the study of chemical and other agents in the workplace, worker exposures, safety and health, and standard setting. Industrial Hygiene covers a very similar terrain.

Regulatory Toxicology: Focuses on ways in which humans and the environment can be protected from toxic effects, through regulations and standard setting. Considers scientific decision-making within a societal and legal framework. Relies heavily upon risk assessment.

Toxicogenomics: Concerned with the compilation and synthesis of information regarding gene and protein expression in order to understand molecular mechanisms involved in toxicity. Toxicogenomics calls upon proteomics, metabolomics, and transcriptomics to identify biomarkers that predict toxicity and genetic susceptibility to harmful substances. Environmental pollutants, pharmaceuticals, and other potentially toxic substances are all within the scope of toxicogenomics research.

Computational Toxicology: Deals with the use of modern computational approaches and information technologies to elucidate mechanisms of toxicity. May also be referred to as toxicoinformatics.

Virtually every branch of toxicology listed overlaps with at least one other. Other ways to parse the discipline are by agents under consideration, such as venoms, pesticides, metals, solvents, drugs, and radiation. One can also look, instead, at target biological systems which the agent may affect, for example, liver, kidney, skin, and heart. As for toxins, they can be categorized by their biological origin, such as insect-, plant-, reptile-, or marine-derived toxins. Some toxicologists spend their careers focused very tightly on a subject, while others graze across many research fields.

ABOUT HISTORY

History is *about* the past; it is not the past. The past is passive, objective, all encompassing. History is active, subjective, and selective. The further back in time that we look, the more problematic it is for us to reach, in the present, conclusions about what happened in the past. Examples, particularly from ancient eras, described below, will show how tales accepted without question are currently being re-examined and revised, and remind us that history is also relative.

Science begins with observation. In the distant past, our observational skills did not extend beyond our senses. We put our senses, to good use, nevertheless, in assessing toxicity and safety even in prehistorical times (i.e., before the written record). Our hominin ancestors used trial and error extensively to explore their environment. In terms of toxicology, they would make careful note of which substances, particularly potential food sources, were safe and which were hazardous. Although it might very well be after the damage was done, they and their tribe and descendants would quickly learn to differentiate between the safe and toxic. Toxic substances, of course, were to be avoided, although it soon became clear that they could be used against enemies.

There are numerous ways to approach the history of toxicology because there are many histories, such as those of the branches outlined in the previous section. Complicating the presentation of a uniform history is the fact that these individual histories overlap. Given the space limitations of this chapter, we will focus on chemicals and proceed chronologically, taking occasional detours as necessary.

TOXICOLOGY IN ANTIQUITY

Ancient China

Shen Nong, the legendary founder of Chinese Herbal Medicine, also known as the farmer god (for he also taught his people how to farm), and said to live circa 2800 BC, saved his subjects from the worry of trying different potential food plants to decide whether they were poisonous. He was said to have tasted hundreds of herbs daily to differentiate the poisonous from the medicinal or just plain edible. Although the toxins he encountered made him sick frequently, he somehow survived them. He is also considered the author of perhaps the world's first pharmacological compendium, *Divine Farmer's Classic of Materia Medica*. His text, a compilation of oral

traditions, was compiled in the 3rd century AD. Legend also has it that Shen Nong discovered tea when, sitting under a Camellia tree, dried leaves fell into the water he was boiling to drink (Wilkinson, 2007; Yang, 1998).

Du (毒) is the standard word for poison or toxicity in Chinese. It was understood by the ancient Chinese that drugs (herbals in this instance) were potentially toxic and dose played a role. Aconite, derived from the plant wolfsbane and possessing extreme potential toxicity, was widely used medicinally in small doses in China over 2000 years ago. It was usually applied externally, often processed in some way or mixed with other drugs, to treat various wounds, but was also ingested as a tonic to restore qi (the vital energy defined by Chinese medicine) and extend life. At the same time, sources from that era show that unadulterated aconite in larger doses was often used to murder (Liu, 2014). Today we know that the alkaloids in aconite have a narrow therapeutic index and their use is not generally recommended. Interestingly, it took several thousand years for the role of dose in toxicity to be firmly articulated in the West by Paracelsus, who is discussed later in this chapter.

The ancient Chinese poison, Gu, is one of many potions residing in that blurry historical space between fact and legend. Presumably, a variety of venomous creatures such as snakes, lizards, scorpions, and insects were confined in a container and left to devour each other until only one was left. This survivor thus concentrated in its body the toxins of all its former cell mates and the venom extracted from it was believed to be superbly potent.

Ancient India

Ancient India was no stranger to the knowledge and uses of poisons. Poisoned weapons of various sorts were well known. A Sanskrit verse reads, "Jalam visravayet sarmavamavisravyam ca dusayet," or "Waters of wells were to be mixed with poison and thus polluted" (Khajja et al., 2011). Sushruta was an Indian surgeon. Volume 5 of his medical and surgical compendium, *Suśrutasaṃhitā*, a foundational work in Ayurveda (traditional Indian medicine), contains several chapters related to poisons and poisoning, including descriptions of vegetable and mineral poisons (Sthavara) and animal poisons (Jangama), as well as advice on medical treatment of snake bites and insect bites (Wisdom Library, n.d.). *Agada Tantra*, one of the eight clinical specialties of Ayurvedic medicine, is specifically associated with toxicology (Manohar, 2014; Wujastyk, 2003).

India also has a long tradition of tales about the so-called "venomous virgin" (visakanya), first mentioned in the *Suśrutasaṃhitā*. This maiden, sometimes referred to as the "poison damsel," would, as a young girl, be fed "tolerably minute, but gradually increasing, amounts of poison or snake venom, and that by the time she was an attractive young woman, the level of toxin in her body would be so high that she could be sent to an enemy king as a gift. Upon kissing her, making love to her, or even just sharing glass of wine with her, he would instantly fall dead" (Slouber, 2015). The Rig Veda itself, one of the four texts sacred to Hinduism, includes hymns related to poisons (Wikisource, n.d.-a).

Ancient Egypt

Ancient Egypt was for nearly 30 centuries one of the world's preeminent civilizations and has left us a legacy of unrivalled art, architecture, and religious traditions. Animals played an important role in its belief systems. Egyptian gods and goddesses often took on a hybrid human–animal physical form.

Venomous snakes and insects were well known and the focus of toxicology as it existed in ancient Egypt. One of the major documents examining snakebite, and surviving in most of its entirety to our time are the Brooklyn Papyri (held by the Brooklyn Museum), 525–600 BC (Sanchez and Harer, 2014). Its two sections describe individual snakes and treatment for snakebites, respectively. Paragraph 15 of the Papyri, for example, describes the snake known by the Egyptians as Apophis which, mythologically, personified evil. Scholars believe this may be the Boomslang (*Dyspholidus typhus*) in the Colubridae family. Symptoms and signs of snake envenomation are presented in the Papyri. The treatments offered could be general, for any snakebite, or specific. Bites by snakes known to be lethal generally received no treatment. Therapeutic measures, overall, were largely symptomatic. One treatment that comes up with frequency is the use of Allii Cepae, the onion, used in various preparations depending on the bite. Often this was used in conjunction with induced vomiting to rid the body of the poison:

Paragraph 41: Very good remedies to be made for those suffering from all snake bites: Onion, ground finely in beer. Eat and spit out for one day. (then follows an incantation)
Paragraph 42: As for the onion, it should be in the hand of the priest of Serqet, wherever he is. It is that which kills the venom of every snake, male or female. If one grinds it in water and one smears a man with it, the snake will not bite him. If one grinds it in beer and sprinkles it all over the house one day in the new year, no serpent male or female will penetrate therein. (Nunn, 1996)

Toxicity is addressed to a lesser extent in other important papyri such as the Berlin, Edwin Smith, and Ebers papyri.

Cleopatra VII, born in 69 BC, is one of the most fascinating personalities to flourish in Egypt when Greece and Rome held sway. During her reign as Pharaoh, Egypt was a Hellenistic (i.e., Greek) province, part of the Ptolemaic dynasty, established after the death of Alexander the Great. After Cleopatra's death, Egypt was annexed by Rome. And while her romantic exploits with Julius Caesar and Mark Anthony have been grist for generations of writers and artists, it is her death that holds toxicological interest for us. After the Battle of Actium (on Greece's west coast), which ended in defeat for the Egyptians, and learning that Marc Anthony killed himself by a self-inflicted sword wound, Cleopatra decided to follow suit. It is said that she had her servants bring her a basket of figs, in which one or more asps (Egyptian cobra) were hidden, and holding one to her breast, she succumbed to its venomous bite. A recent analysis questions the feasibility of a maid capable of carrying a basket of one or more Royal Cobras (9.8–13 ft in length, and weighing some 13 lbs) camouflaged by figs (Tsoucalas and Sgantzos, 2014). Other evidence on the time frame of her dying support this doubt. It has now been suggested that a more likely scenario was that she was murdered, perhaps with a poisonous draught by Octavian, the victor in their battle. He may have then spread the rumor of her suicide to avoid turmoil in the streets (against him) by the subjects who adored her.

Pontus, Mithridates, and Theriacas

The kingdom of Pontus in northeastern Turkey played an interesting role in the history of poisons and antidotes. Mithridates VI, its ruler beginning in 120 BC, was a fierce adversary of Rome, engaging it in battle three times. Ultimately, he succumbed to defeat by Pompey in the third war and committed suicide. Even as a boy, Mithridates experimented with poisons and antidotes, even on himself. Son of a father who was murdered with poison and a mother who would have poisoned him in order to ascend to the throne, he went into hiding for a period of years. He returned to capture his rightful position by likewise using poison, probably arsenic. With a background like that, one could hardly consider it paranoia that he feared assassination by poison and took precautions to avoid it (Mayor, 2010).

His approach was to ingest small doses of toxicants to become immune to them. His lifelong pursuit was to create a universal antidote, which came to be known as a theriac, his particular one called a Mithridatium, by creating a concoction of tiny amounts of deadly poisons and antidotes. Not as far-fetched as it seems, recent science reveals that exposure over thousands of years to arsenic among certain Andean highland populations may have resulted in a level of resistance in their modern-day descendants (Schlebusch et al., 2013).

There have been many speculations about what the ingredients of the Mithridatium were, but we do not know for certain, and may never know. Returning to Mithridates' defeat by Pompey, legend holds that the ignominy of it led him to want to end his life. He retreated, with a poison, to the highest tower of his castle with his daughters. His daughters insisted that they be administered the poison first. After they died, he drank the balance. He weakened, but did not die, and his disorientation prevented him from stabbing himself with his own sword as he attempted. Instead, at least in one version of his actual death, he appealed to his bodyguard, Bituitus, to impale him with a sword.

Ancient Greece

Nicander of Colophon (fl 130 BC), a Greek poet and physician, is the author of two of the oldest extant works on poisons—*Theriaka* and *Alexipharmaka*—both written in hexameter verse (Gow and Scholfield, 2014; Touwaide, 2014b). The *Theriaka* concerns venomous animals. As such they have a delivery system through which injection of their venom can be harmful to humans and other organisms. A large portion of this volume is devoted to snakes. Among other information, he describes 15 snakes, including several cobras, and the symptoms in humans associated with envenomation, followed by discussion of remedies. Additional narrative is devoted to spiders, scorpions, insects, lizards, and fish. His *Alexipharmaka*, a briefer poem, deals with 21 poisons from the vegetable, mineral, and animal kingdoms. Among them are aconite, white lead, and hemlock. As in his companion work, Nicander describes the poison, its symptoms, and antidotes.

The Greek philosopher, Socrates (469–399 BC), whose wisdom was kept alive through the ages via his disciple, Plato, became an iconic figure in the history of toxicology through his death. Convicted of corrupting the youth of Athens and disrespecting the gods, he was sentenced to death. The received knowledge of the ages,

historiographically transmitted, is that his execution was to be carried out in suicidal fashion, with Socrates condemned to drink an extract of hemlock, a poisonous plant (*Conium maculatum*) well known to the ancients. Recently, scientific evidence has called this into question largely because the account provided in Plato's *Phaedo* describes a clinical disorder not caused by hemlock poisoning (Dayan, 2009), although the debate has yet to be resolved and some sources point to a possible mixture of hemlock and opium (Arihan et al., 2014).

Alexander the Great (born 356 BC) plays a role in the history of toxicology in Greece in that the cause of his death is an unsolved mystery as well (Mayor, 2014). He is said to have drunk vast quantities of wine at a banquet in Babylon, after which he suffered severe abdominal pain. Over days, things went from bad to worse and he developed partial paralysis finally dying two weeks later. Rumors of poisoning began circulating in no time. He had enough enemies. Some even thought that Aristotle, his former tutor, poisoned him. Some of his friends guessed that he succumbed to a legendary poison taken from the waterfall of the Styx River, not only the mythological entrance to Hades, but an actual place in the north central Peloponnese. Ancient writers have considered the river poisoned. Though possibilities abound and speculation is widespread, the true cause of Alexander's death has never been confirmed.

Recent discoveries suggest that even the Oracle at Delphi, perhaps the most important and sacred shrine in ancient Greece, is, in a curious fashion, toxicologically significant. Associated with the Greek god Apollo, people would pilgrimage to Delphi with questions usually about what events would occur in the future. They would address their questions to the Pythia, a role filled by various women at different time. Plutarch, the celebrated Greek biographer and essayist, served as one of the priests at the temple of Apollo at Delphi. He noted that *pneuma* (a kind of gas or vapor) was emitted in the *adyton*, a small inner sanctum type area (de Boer, 2014). The Pythia would sit on a tripod-shaped chair, given a chance to inhale the *pneuma*, and go into a trance, after which a priest would address to her the questions asked by the petitioners. Similar accounts appear in ancient texts by others including Plato. Modern-day research attempted to assess the likelihood of an actual gas affecting the mental states of these priestesses. A 2002 paper bringing together the skills of a geologist, archaeologist, and clinical toxicologist reviewed the various research studies, concluding that "the probable cause of the trancelike state used by the Pythia at the oracle of Delphi during her mantic sessions was produced under the influence of inhaling ethylene gas or a mixture of ethylene and ethane from a naturally occurring vent of geological origin" (Spiller et al., 2002).

Toxicology is also heir to a rich mythological tradition. After Hercules, for example, killed the nine-headed sea monster known as the Hydra, as part of his second labor, he cut it open and dipped his arrows in its venom, providing him with what may have been the first biological weapon for use in future battles. Achilles, one of the prominent heroes in Homer's *Iliad* was a victim of just such a poison. Immersed as an infant in the river Styx by his mother to make him immortal, she failed to realize that in holding him by the heel, that very part of the body would make him susceptible to future danger. And so, it was that in the final battle of the Trojan War, he was killed by a poisoned arrow shot into this heel. These are but two examples of how poisons were incorporated into myth and legend in ancient Greece and elsewhere.

Ancient Rome

The Romans of antiquity were also knowledgeable in the principles and practice of toxicology. Interestingly, the Latin word *venenum* can mean either poison or remedy, and one would typically modify the term according to the usage intended (i.e., *bonum venenum* or *malum venenum*).

Dioscorides (born 40 AD), a native of Anazardus, Cilicia, Asia Minor, was a physician who traveled through the Roman Empire with Emperor Nero's army. He would collect samples of local medicinal herbs as he encountered them. The information he gleaned became material for his encyclopedic *De materia medica*, compiled in the 1st century AD, and relied upon for centuries as the most extensive and reliable herbal available. In it he classified poisons as animal, plant, or mineral (Timbrell, 2005). More specifically, *De Venenis* and *De venenosis animalibus*, ascribed to Dioscorides but probably not written by him, covered poisons in general and animal venoms, respectively, and were very influential works in toxicology down through the ages (Touwaide, 2014a).

Galen, another Roman Empire era physician, born (129 AD) in Pergamon, had a monumental impact on the understanding and practice of medicine. He became court physician to Marcus Aurelius. He was a firm subscriber to the theory of the humors (blood, yellow bile, black bile, and phlegm), the origins of which may go back to ancient Egypt but which were first articulated about medicine by Hippocrates. Galen formulated his own Galeni

Theriaca and claimed it improved upon the one concocted by Mithridates (Karaberopoulos et al., 2012). He wrote about assorted theriac compounds in his books *De Antidotis* I and II and *De Theriaca ad Pisonem*. Indeed, he tested them by bringing roosters into contact with snakes.

Poisoning, especially among the ruling classes, was frequently practiced, typically (but not exclusively) by women upon their husbands or other inconvenient relatives. If they did not have the skills to do the deed themselves, they sought professional poisoners, usually women as well. One of the most notorious of the lot was Locusta. As the story is told, she was summoned by Agrippina, the wife of Emperor Claudius, to kill him so that Agrippina's son, Nero, from a previous marriage would become the new Roman emperor. Locusta supplied Agrippina with a batch either of poisoned or poisonous mushrooms. Though taken quite ill, the mushrooms did not kill Claudius outright. Quick thinking (though history is not quite clear by whom) led Agrippina to convince Claudius to let her run a feather down his throat to expel the poison. The feather itself, though, was coated with a lethal dose of poison which killed Claudius and thus Nero assumed the throne. Though Locusta was imprisoned, it was not long before Nero had her released and, in fact, employed her to poison Britannicus, a son of Claudius from a previous marriage and thus a threat to the new emperor. Nero ultimately pardoned Locusta for all past crimes and she was allowed to establish a school to train others in her art.

The legal framework of toxicology is sometimes dated back to the age of the Roman military and political leader Sulla. Under the *lex Cornelia de sicariis et veneficis* (81 BC), punishment was imposed for anyone who prepared, sold, bought, kept, or administered a noxious poison (venenum malum) (Hobenreich and Rizzelli, 2014).

A theory proposed in 1983 by Jerome Nriagu popularized the idea that the metal lead was responsible for the fall of the Roman Empire. It has been stated that the ruling classes, in particular, were exposed to lead contamination in water supplies, cooking, and the production of wine, ultimately decreasing their fertility and reproductive capacity. More recent archaeological investigations have found that although clinical lead poisoning probably did occur, the mean skeletal lead content of populations at the time was less than half that of present-day Europeans in the same regions. The assertion that lead was the primary culprit in Rome's decline and fall has been largely refuted (Cilliers and Retief, 2014a, 2014b).

Lead has continued to plague mankind, in occupational and other exposures, through the ages. Interestingly, in 1921 a global treaty the White Lead (Painting) Convention was adopted. It was meant to largely prohibit the use of white lead as a pigment in paint. With no thanks to the Lead Industries Association, this was never ratified by the United States (Hernberg, 2000). Herbert Needleman, a physician, was instrumental in helping us understand how lead affects children, particularly with his 1979 study in the *New England Journal of Medicine* noting deficits in children with high dental lead levels (Rosner and Markowitz, 2005). Still a concern in inner cities, lead periodically makes the headlines, as in the case of its seepage into the drinking water of Flint, Michigan, in 2016.

THE MIDDLE AGES AND RENAISSANCE

As we transition from antiquity to the Middle Ages at about 400 AD, toxicology continues to have a presence in European society vis-à-vis both poisoning as a means of dispatching enemies but increasingly in trying to establish its scientific foundation. Some of the well-accepted tenets of the toxicology of this time such as the hypothesis that the saliva of rabid dogs was a poison on a par with snake venom would see revision, but the scientific method was at least beginning to take hold.

The Venetian Council of Ten was a governing body in Venice from around 1310 until 1797. They were known for conducting secret tribunals whereby figures perceived as a threat to the state were ordered executed. Many of these executions were carried out by poisoning. There were several attempts on the life of Francesco Sforza of Milan, while Mehmed II, Sultan of the Ottoman Empire, was allegedly ordered to be poisoned by the Council (Jutte, 2015).

Poisoners continued to find steady employment but some reputations, as will be seen in the following paragraphs, were ill-deserved. Poisoning as an assassination method was widespread during the 14th to 16th centuries in Europe. Letters to Grand Duke Cosimo I de'Medici affirm as much. Animal venoms, phytotoxins, and mineral poisons were all employed. Cosimo himself was suspected of poisoning and was in possession of a

poison recipe among his confidential documents and his library contained several books in which poisons were discussed. He was also involved in a plot to assassinate Piero Strozzi, part of a rival banking empire, by poisoning his wine. Poisoning was clearly a family affair with the Medicis, and Cosimo's sons Ferdinando and Francesco were equally complicit in it. Despite persistent rumors that Francesco and his wife, Bianca, were poisoned with arsenic by the former's brother, Ferdinando, the official cause of death was listed as malaria. Although recent forensic examinations still do not entirely agree, it now appears most likely that malaria was indeed the culprit (Fornaciari and Bianucci, 2010). Many legends surround Catherine de'Medici who moved to France to marry the future King Henry II. Despite multiple purported victims, there is no definitive evidence that she poisoned anyone. Developing and testing antidotes was also part of the Medicis' stock-in-trade (Pratte et al., 2014; Barker, 2017).

Another powerful and infamous Italian family, originally from Spain, and on whom were pinned numerous heinous crimes, poisoning among them, were the Borgias. There were claims, for example, that Cesare murdered a servant who was a lover of his sister, Lucretia, in front of their father Pope Alexander. Cesare was also said to have poisoned Cardinal Juan Borgia. The reputation of Lucretia herself was stained with allegations, by enemies of the Borgias, that she was a poisoner. Documents uncovered recently in the Vatican archives refute these and other claims concerning the Borgias and it is now thought that, though saints by no means, their undeserved reputation for extensive poisonings and murders stems from rumors spread and repeated by their enemies (Dal Bello, 2012; Cobb, 2017).

In 17th century France, during the reign of Louis XIV there had been a series of poisonings which have not, at least to date, been subject to any of the above revisionism. It became known as *L'affaire des poisons* (the Affair of the Poisons) and originated with the trial of Madame de Brinvilliers, convicted of poisoning her father and two brothers and attempting to poison other family members. Prior to her execution she implicated, without specifically naming them, many others, who were subsequently prosecuted and sentenced to death. One of the most notorious was the celebrated Catherine Deshayes, also known as La Voisin, an acknowledged sorceress, who did a very good business in poisons, abortions, and black masses. La Voisin was finally burned at the stake in 1680 for her crimes (Duramy, 2012; Somerset, 2014).

Giulia Tofana was yet one more notorious 17th century Italian poisoner, thoroughly skilled at her trade. It is thought that two women in Palermo, Francesca la Sarda and Teofania di Adamo, jointly concocted and marked a poison known as "Acqua Tufania" for which they were executed. Some of their associates fled to Rome and, under the leadership of Giulia Tofana, possibly Teofania's daughter, they carried on the business, even after the death of Giulia. The poison became known as Aqua Tofana. Arsenic was likely a primary ingredient. It was sold throughout Italy to domestically unsatisfied women seeking freedom from their husbands. Aqua Tofana became an almost generic term for particularly potent poisons and the term has appeared in various sources, including medical textbooks, for some two centuries. Although originally producing violent symptoms, it ultimately became associated with a class of toxicants known as "slow poisons," which rather than existing in fact may have simply been a speculative class of agents designed to fuel the imaginations of the easily swayed (Dash, 2015).

As already mentioned, the Middle Ages and Renaissance were times not only of commonplace poisonings, particularly among the aristocracy and ruling classes, but of an increasingly sophisticated understanding of toxicology. Moses Maimonides, the great Jewish philosopher, theologian, and scientist, wrote his *Treatise on Poisons and their Antidotes*, originally in Arabic, in 1198. Part I was concerned with bites from snakes and rabid dogs (toxicology, remember, was still in its formative stage), and stings of scorpions and insects. Part II dealt with poisons in food and minerals, as well as remedies. He made a distinction between "hot" and "cold" poisons which, it has been claimed, may be equivalent to modern-day hemolysins and neurotoxins. Maimonides also emphasized preventive measures (Rosner, 1968; Furst, 2001; Maimonides, 2009).

The study of toxicants was so widespread in Persian and Arabic countries during the Middle Ages that the era has come to be known as the golden age of medieval toxicology. Among prominent toxicologists who wrote noteworthy treatises on the subject were Jābir (Jaber) ibn Hayyān (721–815 AD), Ibn Maāsawyah (Yuhanna ibn Masawyah, Abu Zakariya, 777–857 AD), and Ibn Wahḫshīyah al-Nabti (9–10th century AD). Known by his Latin name of Avicenna in the West, Abū ʼAlīAal-Ḥusayn ibn Abd Allāh ibn Sīnā was perhaps the most noteworthy physician/scientist/philosopher of the Islamic world. His celebrated "Canon of Medicine" remained the most popular medical textbook for some six centuries (Nasser et al., 2009). Covering a broad range of topics, it includes detailed descriptions of venoms and other poisons, such as opioids and oleander, as well as instructions related to

antidotes (Ardestani et al., 2017). He even explored the effect of alcohol on opium poisoning:

Patients may have concurrent alcohol poisoning. It can have a synergistic effect with opium poisoning and decrease its lethal dose. On the other hand, alcohol may serve as an opium antidote. This effect depends on the amount of ingested alcohol.

Many of his observations have been confirmed by current medical knowledge (Heydari et al., 2013).

On a very practical level, as was seen even in the Roman era, it became clear to ordinary people, especially those whose work entailed significant exposure to certain natural materials such as minerals, that their very occupations could be harmful. Georgius Agricola (1494–1555) born in the kingdom of Saxony, currently part of Germany, studied many subjects and completed his medical education in Padua. He has come to be known as "the father of mineralogy" largely as a result of his best known monograph, *De Re Metallica*, published in 1556.

Inevitably we reach the point where we address the incalculable contributions of the unorthodox medical revolutionary, Theophrastus von Hohenheim, called Paracelsus (1493/94–1541). Born in Einsiedeln, a municipality now in modern-day Switzerland, he was a wanderer and iconoclast, and strongly tied to the alchemical tradition. He theorized that there were four pillars of medicine: natural philosophy, astronomy, alchemy, and medical virtue. He went his own way and was not highly regarded by the medical establishment or local government officials. Indeed, as a lecturer at the University of Basel (as well as the city's municipal physician until being forced to flee), he burned the standard medical textbooks of the day, such as those of Avicenna and Galen (Borzelleca, 1999). History, though, has vindicated many of his teachings. In addition to his medical works, he was a keen observer and investigator of toxic effects of various agents and wrote a treatise about their effects upon miners. He concludes this work with a discussion of metallic mercury and criticizes its use at the time as therapy for people afflicted with syphilis (Gantenbein, 2017).

The most famous toxicological adage associated with Paracelsus is "The dose makes the poison," which is a distillation of what he wrote in his *Seven Defenses*, designed to defend his controversial teachings in the face of his adversaries:

Wenn jhr jedes Gifft recht wolt außlegen/ Was ist das nit Gifft ist? alle ding sind Giffi/ vnd nichts ohn Giffi/ allein die Dosis macht/ dz ein ding kein Gifft ist. When you want to correctly evaluate a poison, what is there that is not poison? All things are poison and nothing is without poison; only the dose determines that something is not a poison.

This was surely known in various and sundry ways, certainly by experience, long before the time of Paracelsus, but never had it been so well articulated. We may, today, look upon the latter portion of this statement as an oversimplification. After all, what about factors other than dose which influence toxicity—gender, age, pre-existing conditions, genetics, the microbiome, etc.? This is all well and good, and it is not unusual for quite valid eureka moments to be refined over time, but for a concise encapsulation of one of the key components of what and when something is a poison, and which continues to serve as a bedrock of toxicology, Paracelsus deserves the laurel crown and the oft-cited appellation, "Father of toxicology." An understanding of the dose–response relationship is no less significant to our understanding of toxicology today than it was 500 years ago.

It is tempting to declare Paracelsus' legacy as ironclad. However, proponents of a theory originating in the 19th century known as hormesis are today suggesting that substances known to be toxic at elevated doses may actually have a beneficial effect at very low doses. Non-monotonic dose–response (NMDR) curves graphically describe hormesis. Hormesis remains a controversial theory among toxicologists.

Paracelsus was but one example of the tenuous link between alchemy and toxicology. The alchemist Jan Baptist Van Helmont, though once a disciple of Paracelsus, ultimately went his own way. Van Helmont did acknowledge that almost everything in nature is possessed of some secret poison but that somehow it overlay a core of goodness. He referred to the bible and medical alchemical theories to support his views and reveal ways to remove the poison (Hedeson, 2017).

Other key figures were Pietro d'Abano who compiled a treatise devoted to poisons and their remedies, *De venenis*, which sought to return to the pure Greek roots of toxicology; the Paduan physician Girolamo Cardano who offered a careful analysis on the relationship between poison and putrefaction; Gerolamo Mercuriale who focused on reconciling ancient and contemporary definitions of poison; and Andrea Bacci who argued against a universal definition of poison and also said that its unusual powers made it similar to other natural substances such as the magnet (Gibbs, 2017; see http://fredgibbs.net/posts/universals-and-particulars-of-poison).

Interest has always been keen on both preventing and treating poisoning. Various products of biological origin, typically solid and hard, were said to serve in this capacity. They include stones, shark teeth, bezoars, and

horns, sometimes embellished and worn as jewelry, and used in table settings or even in some instances found in graves. A bezoar stone is an indigestible mass found in the gastrointestinal system, especially the stomach. Etymologically, the word derives from the Farsi words, *bāk* (purification) and *zahr* (poison) and, indeed, the stones were described in ancient Arabic medical literature since the 8th century and used as antidotes by Persian, Arab, and Jewish physicians. Belief in bezoars made its way to Europe and is mentioned in Johannes de Cuba's *Hortus Sanitatis* in 1485 and Pietro d'Abano described their use in 1565 (Barroso, 2014, 2017).

Fossil shark teeth (Glossopetrae), as well, have found application as prophylactics, detectors, and neutralizers of poisons. In medieval times, it was said that such teeth mounted in silver announced poisons by "sweating" or changing color. Their ability to detect poison and protect humans from poisoning is cited in Lapidaries such as those of Marbode (11th century), Sloane (16th century), and Jean de Mandeville. Miocence specimens of *Otodus megalodon* from Malta were said to be the most efficacious of the shark's teeth. Due to a 16th century shortage of bezoar stones, a substitute that came to be known as Goa Stones was formulated. In addition to various precious stones, coral, ambergris, and musk, they often contained pulverized fossil shark teeth. Often gold-plated, they could be housed in containers of elaborate silver or gold. Scrapings from these stones mixed in wine, beer, or other beverages could purportedly ward off the effects of any poisons (Duffin, 2017).

Alicorn, that is, the horn of the mythical unicorn, was thought to have medicinal and poison detecting qualities. By the end of the 14th century, the idea became established that it too like shark teeth could detect poison by perspiring in the presence of adulterated food and drink. One of the earliest medieval sources about the medicinal power of unicorns (though the horn per se is not mentioned) is the *Physica by* Hildegard of Bingen (1098–1179) (Lavers, 2017). James Primrose noted: "It can scarce be said, whether to the Bezaar stone, or to the Unicornes horn the common people attributes greater vertues, for those are thought to be the prime Antidotes of all" (Primrose, 1651). Narwhal teeth or the horns of many another animal were likely passed off as unicorn horns. In 1389, John of Herse made a pilgrimage to Jerusalem and observed, "Near the field Helyon in the Holy Land is the river Mara whose bitter water Moses struck with his staff and made sweet so that the children of Israel could drink thereof. Even now evil and unclean beasts poison it after the going down of the sun; but in the morning the unicorn comes from the sea and dips its horn into the stream and thereby expels the poison so that the other animals can drink of it during the day. The fact which I describe I have seen with my own eyes" (Unitarian Review, 1879). There was not, though, universal acceptance of the anti-toxic legitimacy of unicorn products (including powder). Two respected French authorities, Ambroise Paré (1510–1590), court physician to four French kings, and the pharmacist Laurent Catelan (1568–1590) from Montpellier, had differing views on alicorn, with the former a detractor of its efficacy and the latter a proponent (Gerritsen, 2007). Eventually, as with much else, the antidotal property of unicorn horns was consigned to legend.

18TH AND 19TH CENTURIES

Hermetical traditions such as alchemy did not suddenly disappear come 1700. Isaac Newton himself was a passionate alchemist, as was Robert Boyle, often considered the father of modern chemistry. That said, the scientific method gained increasing prominence in the 18th and 19th centuries as a way of understanding our universe, and toxicology benefited from this more sophisticated and methodical approach. A number of scientists made important contributions to toxicology during this time.

Richard Mead (1673–1754) is the author of the first book in English devoted solely to poisons, *A Mechanical Account of Poisons in Several Essays*. He described the signs and symptoms of snake envenomation, performed chemical tests on venom, and experimented on snakes (to study their venom delivery system) and other animals (Seifert, 2011).

Bernardino Ramazzini, born in Carpi, Italy, and educated at the University of Parma, was a physician whose seminal achievements have earned him the moniker Father of Occupational Medicine (Pope, 2004). While the connection between workers' illnesses and their workplace environment, including materials to which they are exposed, had been noted by the ancients, Ramazzini's analysis of this linkage raised the issue to an entirely new level. The first edition of his most famous book, *De Morbis Artificum Diatriba* (*A Treatise on the Diseases of Workers*), published in 1700, is the first comprehensive and systematic work on occupational diseases (Felton, 1997). It outlined the health hazards of chemicals and other substances, including repetitive motions, encountered

by workers in over 50 occupations. Among Ramazzini's many enlightening observations, and one in which he quotes Hippocrates, is the following:

"When you come to a patient's house, you should ask him what sort of pains he has, what caused them, how many days he has been ill, whether the bowels are working and what sort of food he eats." So says Hippocrates in his work Affections. I may venture to add one more question: what occupation does he follow?

The spirit of Ramazzini lives on in the Collegium Ramazzini (CollegiumRazazzini), an independent, international academy founded in 1982 by Irving J. Selikoff and others, to advance the study of occupational and environmental health issues. It holds conferences, symposia, and training courses, and publishes statements and research papers.

Another key figure in occupational toxicology is Percivall Pott (1714–1788), born in London. In 1774 he published an essay, *Chirurgical Observations Relative to the Cataract, the Polypus of the Nose, the Cancer of the Scrotum*. In this he made the link between the profession of chimney sweeps (regarding soot lodging in the folds of scrotal skin) and scrotal cancer (Brown and Thornton, 1957). This was the first occupational link to cancer and Pott's investigations contributed to the science of epidemiology. It wasn't until the 1920s that benzo[a]pyrene was identified as the actual chemical responsible (Dronsfield, 2006).

There were many scientists spanning the 18th and 19th centuries who played significant roles in making toxicology the discipline that it is. The ability to synthesize new chemicals and the added ability to detect their presence, especially in small amounts, marked the beginning of the modern era of toxicology. For centuries, poisonings were confirmed only by confession or eye witness accounts. Making the leap from merely suspecting adulteration or poisoning to irrefutable proof was a major milestone for toxicology. Four scientists who made remarkable advances in the area of chemical detection were Karl Wilhelm Scheele, Christian Friedrich Samuel Hahnemman, Johann Daniel Metzger, and Valentine Rose. Scheele discovered oxygen before Joseph Priestley, although he published his results later. He is also credited with the discovery of hydrofluoric, hydrocyanic, and arsenic acids, and devised methods for detecting arsenic in body fluids and corpses. Hahnemman discovered a test for arsenic oxide. Rose and Metzger discovered the first methods for detecting elemental arsenic and arsenic oxides in fluids and tissues (Farrell, 1994). In 1836, the English chemist James Marsh developed what came to be known as the Marsh test, a groundbreaking method for detecting arsenic.

The medical celebrity Mathieu Joseph Bonaventure Orfila (1787–1853) is often claimed by Spain (where he was born and studied) and France (where he continued his studies, worked, and died) (Bertomeu-Sanchez and Nieto-Galan, 2006; Bertomeu-Sanchez, 2009). While very influential in applying the concepts of chemistry to medicine, it was in toxicology that he excelled and for which he is best known. He became Dean of the Paris Medical Faculty and was a founding member of the Academy of Medicine. At a time when animal experimentation was somewhat less frowned upon, he experimented widely with dogs, varying the amount of poison (such as arsenic) administered and the route of administration, and tested antidotes and treatments. He authored *Traite des poisons*, one of the most popular textbooks of the first half of the 19th century (Orfila, 1814–1815). He subsequently extracted the sections on antidotes and treatments and published them in a compact free-standing volume designed not only for physicians but also for lay audiences that may not have access to medical care but need to know what to do in the event of a poisoning emergency.

Orfila was called to act as a medical expert in various criminal cases. He is best known for a case involving Marie Lafarge, charged with poisoning her husband. Eyewitnesses had seen her buying arsenic (used to exterminate rats) and stirring a white powder into her husband's food. Upon his exhumation, no evidence of arsenic was found using the newly improved test for arsenic devised by James Marsh, although doubts remained whether the physicians were performing the test properly. Orfila was summoned and found definite traces of arsenic in the body, and demonstrated that it did not come from the surrounding soil. Marie Lafarge was found guilty of murder and received a death sentence, later commuted to life in prison. The case cemented Orfila's reputation as the greatest toxicologist of the day.

And yes, indeed, not only Paracelsus, but also Orfila has been called "Father of Toxicology," but of course representing a different era, and for different reasons. "Father of Forensic Toxicology," or "Father of Modern Toxicology," might be more precise. Let's hope that all these "Father of Toxicology" claims don't result in any paternity suits.

In France, Francois Magendie (1783–1855) was best known for his pioneering contributions in neuroscience and neurosurgery, and experimental physiology. His studies on the effects of drugs on different parts of the body

though led to the introduction of compounds such as strychnine and morphine into medical practice (Tubbs et al., 2008). His research into the mechanisms of toxicity of these and other alkaloids furthered the science of toxicology.

Claude Bernard (1813–1878), Magendie's most celebrated pupil, made several physiological discoveries including the role of the pancreas in digestion, the regulation of the blood supply by vasomotor nerves, and the glycogenic function of the liver. His work also led to an understanding of the self-regulating process of living organisms we now refer to as homeostasis. He won acclaim for his book *Introduction à l'Etude de la Médecine Expérimentale* (*An Introduction to the Study of Experimental Medicine*), a classic in the field. He stressed the importance of starting with a hypothesis and having results which are reproducible, thereby furthering the paradigm of the modern scientific method. In the realm of toxicology, Bernard demonstrated that the mechanism of action of curare resulted from its interference in the conduction of nerve impulses from the motor nerve to skeletal muscle. The sensory nerves were left intact. In addition to curare, he studied the toxicological properties of other neuroactive compounds such as opium, atropine, strychnine, and nicotine (Bernard, 1857; Conti, 2002). He was also the first to describe the hypoxic effects of carbon monoxide. Bernard was attuned to how the perturbation of biological systems by toxic agents can be of value to basic science. He stated:

Poisons can be employed as means for the destruction of life or as agents for the treatment of the sick but in addition there is a third of particular interest to the physiologist. For him the poison becomes an instrument which dissociates and analyses the most delicate phenomena of living structures and by attending carefully to their mechanism in causing death he can learn indirectly much about the physiological processes of life …

While Orfila, as we have seen, also experimented on dogs, and was one of many scientists, including Magendie, to subscribe to animal experimentation, Bernard established it as part of the scientific method. He stated:

Experiments on animals are entirely conclusive for the toxicology and hygiene of man. The effects of these substances are the same on man as on animals, save for differences in degree.

Bernard, though an acknowledged seminal figure in experimental medicine, was criticized over his vivisection experiments on unanesthetized animals. The debate over the moral ramifications of animal experimentation gained steam during his lifetime. Interestingly, his wife was appalled by this part of his work. She left him, took their daughters, and with them became ardent anti-vivisectionists (Cavan, n.d.).

Greatly influenced by Orfila, Robert Christison (1797–1882), a Scottish physician, was interested in underpinning medical jurisprudence, especially toxicology, with a scientific foundation. Early on, he investigated the detection and treatment of oxalic acid poisoning and followed this up with investigations on arsenic, lead, opium, and hemlock. His celebrated book, *Treatise on Poisons,* first published in 1829, went through four editions. In addition to his work on poisons, he made important contributions in nephrology (Wikisource, n.d.-b).

Substance abuse, dependence, and addiction have plagued people throughout all time. Published in 1821, Thomas De Quincey's penetrating *Confessions of an English Opium Eater* is an autobiographical account of his opium (more properly laudanum, for he took his opium with alcohol) addiction. His book covers both *The Pleasures of Opium* and *The Pains of Opium*. This may have been the first look at drug addiction but was followed by countless others, fact and fiction, in numerous artistic genres, literary, visual, and even musical: to name a few (some made into movies) Aldous Huxley (*The Doors of Perception*), Hunter S. Thompson (*Fear and Loathing in Las Vegas*), William S. Burroughs (*Naked Lunch* and *Junky*), and Irvine Welsh (*Trainspotting*). Billy Wilder's film, *The Lost Weekend* (1945), featuring Ray Milland, is a classic about alcoholism and Frank Sinatra stars as a heroin addict in *The Man with the Golden Arm* (1955).

THE MODERN ERA

Radiation

The late 19th century is about the time when an understanding of radiation and its potentially hazardous effects began to surface. As is the case with chemicals and biological agents, radiation can be and has been of enormous benefit to society in general and has resulted in countless positive health outcomes via diagnosis and therapy. Nonetheless, precautions are necessary because radiation hazards can be devastating. In 1895, Wilhelm Röntgen

discovered x-rays, electromagnetic energy waves with wavelengths some 1000 times shorter than those of light. He also learned that x-rays could penetrate human flesh. In 1896, Nikola Tesla intentionally exposed his fingers to x-rays and reported burns. In that same year Henri Becquerel discovered that uranium salts naturally emitted similar rays. Marie Curie, a student of Becquerel, named the phenomenon "radioactivity." She went on to discover thorium, polonium, and radium, and received the Nobel Prize twice (once with her husband and Becquerel in physics and later in chemistry). Tragically, her death was attributed to aplastic anemia, likely contracted from her extensive work with radioactive materials (Jorgensen, 2016).

Soon after radium's discovery, it was manufactured synthetically and was believed to have almost magical healing properties. It appeared in food products such as bread, chocolate, toys (because of its luminescence), toothpaste, cosmetics, suppositories, and products to treat impotence. One of the first revelations about the true potency of radioactivity and the scope of its potential danger concerned the unfortunate girls who became radium watch dial painters in the early 1900s. These "radium girls" were hired by the U.S. Radium Corporation to apply radium paint to watch and clock faces so they would glow in the dark. They were instructed to use their lips to shape the brushes to a fine point. By 1927, over 50 women died due to radium paint poisoning, and many of the survivors suffered significant health problems (Mullner, 1999).

The detonation of the world's first atomic bomb in 1945, the Trinity Test, an outgrowth of the Manhattan Project, took place in the New Mexico desert where the nuclear age literally burst upon the scene. There were no doubts, at this point, about the damage such a bomb could inflict and did. On August 6, 1945, while World War II was raging, an American B-20 aircraft dropped an atomic bomb over the city of Hiroshima, killing nearly 100,000 people on impact and decimating virtually the entire city. Maybe half of that number of people were killed when a second atomic bomb was dropped on Nagasaki. Tens of thousands of people in both cities would later die of radiation exposure or otherwise suffer devastating injuries (Blow, 2015). The Treaty on the Non-Proliferation of Nuclear Weapons (NPT), which entered into force in 1970 and was extended indefinitely in 1995, seeks to "prevent the spread of nuclear weapons and weapons technology, to promote cooperation in the peaceful uses of nuclear energy and to further the goal of achieving nuclear disarmament and general and complete disarmament" (UNODA, n.d.).

Although nuclear weapons were developed and used to intentionally wreak destruction and havoc, nuclear power plants are designed to harness the force of the atom for peaceful purposes, that is, to generate energy. However, things do not always go as planned. In 1979, the Three Mile Island plant in Pennsylvania suffered a malfunction that led cooling water to escape from the reactor, and the nuclear fuel rods suffered a partial meltdown. Thankfully, there were no detectable health effects in the population at large. In contrast, the people in the area of the Ukraine where the Chernobyl plant was located experienced a dramatic meltdown in 1986 and were not so fortunate. There was no containment structure and a plume of radioactive material was sent skyward. An estimated 30 people died from radiation poisoning over a period of weeks and several thousand more were put at risk for cancer. In 2011, a massive earthquake and tsunami disabled the power supply and cooling of three Fukushima Daiichi reactors in Japan. All three cores melted within days. No deaths from radiation sickness was reported but over 100,000 people were evacuated from their homes (NPR, n.d.; World Nuclear Association, 2017).

Food and Drugs

The science of qualitative and quantitative chemical detection was applied most effectively to the detection of chemicals in body fluids, drugs, and food. In modern society, we have grown so accustomed to regulations that ensure high standards of purity for most commercial products that it is difficult to remember a time when there were no such protections in place. The realization that there was indeed a need for them evolved gradually. Events leading up to the passage of the Pure Food and Drug Act of 1906 are a good place to start since much of what we consider the modern era of toxicology occurred in and around early efforts to regulate the commerce of food and drugs.

Toxicology has developed and continues, to some extent, to develop as a reactive (rather than proactive) field. Thus, chemical laws and regulations often are enacted in reaction to major or widespread exposure incidents. An early demonstration of this phenomenon is in the efforts to ensure the safety of certain substances to which virtually everyone was exposed, that is, food and drugs. As early as 1848, chemical analyses of agricultural products were carried out in the U.S. Patent office under the Department of the Interior by Lewis Caleb Beck, an American

physician and chemist who researched the adulterants in many drugs commonly prescribed by physicians of the time (Kinch, 2016). In 1846 he published *Adulterations of Various Substances Used in Medicine and the Arts with Means of Detecting Them: Intended as a Manual for the Physician, the Apothecary, and the Artisan*. His publication helped promote the Drug Importation Act of 1848. At the time, there were six major ports of entry within the United States, namely New York, Boston, Baltimore, Philadelphia, New Orleans, and Charleston, where pharmaceuticals entered the American market. The 1848 law required the U.S. Customs Service to inspect and stop any adulterated drugs from entering the U.S. market. Inspectors were typically experienced physicians and pharmacists who could more easily detect a counterfeit substance. They were also armed with the added ability to conduct qualitative tests, such as those detailed in Beck's publication, to determine if a drug was adulterated.

The Department of Agriculture, which would eventually give rise to the Food and Drug Administration (FDA), was established under Abraham Lincoln in 1862. The Division of Chemistry rested within this department and employed a single chemist Charles Mayer Wetherill. In 1883, Harvey W. Wiley, who was to play a highly influential role in safeguarding the country's food and drugs, took over as the Division's fourth chemist. The Division of Chemistry became the Bureau of Chemistry in 1901 and in 1902 Wiley was granted $5000 to administer what came to be called the "Poison Squad" experiments. These experiments involved asking healthy volunteers to consume measured amounts of preservatives routinely added to food items to determine whether they were safe for human consumption. The experiments were carried out in a controlled setting with meals prepared by a designated cook and chemist William R. Carter (Pray, 2003). Although cringeworthy by today's ethical standards, some of the chemicals fed to these young men were borax, benzoic acids, and formaldehyde. While many still question the validity of these sensational experiments, the publicity helped to enlighten consumers about the potential dangers of adulterated foods and the importance of accurate labeling.

Wiley was not alone in his pursuit to rid the market of impure foods and drugs. Journalists as well took up the cause of exposing quack medicines and adulterated food staples thereby fueling Wiley's efforts. The so-called muckraking journalists of the early 20th century exposed hundreds of patent medicines as misleading, harmful, and sometimes deadly. One example (of many) was the case of acetanilide, a nonsteroidal anti-inflammatory drug used to treat pain and reduce fever, but highly toxic. In 1905, Samuel Hopkins Adams published, in *Collier's Weekly*, "The Great American Fraud," a sensational article exposing the hoax of patent medicines (Adams, 1905). Upton Sinclair's 1906 book, *The Jungle*, detailed unsanitary conditions of workers in the meat packing industry. "The Jungle" was published as a serial in 1905 and then as a book in 1906. Despite the many efforts to pass legislation to ensure food and drug safety prior to 1906, nothing seemed to get through both the House and Senate and, unfortunately, many bills languished for years. Wiley worked tirelessly to institute food and drug legislation throughout his tenure at the FDA (1883–1912) and during this time over 100 food and drug bills were introduced in Congress with nearly all failing to gain any traction.

The Pure Food and Drugs Act and the Meat Inspection Act were passed on the very same day in 1906 by the then president Theodore Roosevelt. The former law became known as the "Wiley Bill" due to Harvey Wiley's efforts. The Bureau of Chemistry was reorganized in 1927 into the Food, Drug, and Insecticide Administration, later renamed the Food and Drug Administration and ultimately moved out of the Department of Agriculture entirely and into what is now the Department of Health and Human Services.

To backtrack a bit in time, England's attention to the adulteration of food and drugs actually preceded that of the United States by a half century. Friedrich Accum, Wiley's counterpart in the United Kingdom, published a book in the 1820s titled *A Treatise on the Adulterations of Food, and Culinary Poisons* with the subtitle *There Is Death in the Pot*. Accum wrote about hundreds of poisonous additives commonly used in food products to either sweeten, color, or bulk up foods. He also pointed a finger at the perpetrator, giving the names and addresses of the offending manufacturers, which was unprecedented at the time (Accum, 1820; Oser, 1987). Accum became extremely unpopular among wealthy shop owners and he eventually left the country. Friedrich Accum and, later, Thomas Wakley and Arthur Hill Hassall were the figures most responsible for the campaign to prevent food adulteration which eventually resulted in food and drug legislation in the United Kingdom (Oser, 1987).

The 1906 Pure Food and Drug Act in the United States did not have the broad impact that was intended. Wiley and other supporters were hopeful that the law would have far reaching implications and broadly protect the food supply. However, as written, its main purpose was to ban foreign and interstate traffic of adulterated, falsely advertised, or mislabeled food and drug products. It empowered the U.S. Bureau of Chemistry to inspect products and refer offenders to prosecutors, but gave no prosecutorial power to the agency itself. For example,

during the Jamaican Ginger poisonings detailed in the next paragraph, the FDA was not involved in the investigation or prosecution of the crime until well after the case was resolved by a judge. The law required that the active ingredients be placed on the label of a drug's packaging and that drugs could not fall below purity levels established by the United States Pharmacopeia (USP) or the National Formulary. The USP and National Formulary guidelines were established some years earlier by a group of physicians and pharmacists, and served as a foundation for the Pure Food and Drugs Act. Although the law was popular, it was virtually impossible to enforce. The 1906 law prevented the manufacture, sale, or transportation of adulterated, misbranded, poisonous, or deleterious foods, drugs, medicines, and liquors. The new law led to the establishment of government-run analytical laboratories, and the conditional removal of certain ingredients such as ethanol, herbal mixtures, and coloring agents in most but not all cases. Many sections of the Act were overturned by the then Associate Justice Oliver Wendell Holmes and the U.S. Supreme Court in 1911. Wiley left the Bureau of Chemistry in 1912. The 1906 Act was not perfect, but it was a perfect jump start to the subsequent food and drug reform laws in the United States.

Prohibition in the United States ran from 1920 to 1933. During this time, there were very few legal means for obtaining alcohol. One of the few remaining options for alcohol consumption was via a doctor's prescription which would allow one to procure whiskey or rum from a pharmacist. Meanwhile, it was legal to purchase over-the-counter patent medicines or elixirs containing alcohol. Some disreputable drug companies began increasing the alcoholic content of their medicines or inventing new ones composed almost entirely of alcohol. One infamous concoction was Jamaica Ginger, which contained between 70% and 80% alcohol by weight. The U.S. Treasury Department required changes to the ingredients of Jamaica Ginger to discourage its abuse. The minimum requirement of ginger solids per cubic centimeter of alcohol resulted in a bitter concoction that was not palatable. Inspectors would often boil down the liquid and weigh the solids to ensure that the concoction was formulated appropriately. Two bootleggers (Harry Gross and his brother-in-law Max Reisman) developed an alternative recipe that could pass the inspection and taste well enough to sell by adding tri-ortho-cresyl phosphate (TOCP) to the mixture. In early 1930 reports began to pour in detailing strange paralysis of the legs, arms, and wrists with little to no recovery in large numbers of people throughout the midwest. By 1931 the disease, which had come to be known colloquially as Ginger Jake paralysis, had reached epidemic proportions affecting an estimated 10,000 people across the country from New York to California. Doctors eventually traced the illness back to the Jamaica Ginger elixir, but since the typical ingredients (as listed in the U.S. Pharmacopeia) were not known to cause disease they immediately suspected a contaminant was responsible. The matter was taken up by the Public Health Service's National Institutes of Health (NIH), which was newly formed from the Hygienic Laboratory in 1930. It was there that the adulteration with tri-ortho-cresyl phosphate was discovered. There were over 35,000 members of the United Victims of Ginger Paralysis Association (Morgan and Penovich, 1978). The Ginger Jake episode and other cases of false therapeutic claims made it clear that change needed to come to the 1906 law, and change it did, propelled by the sulfanilamide poisonings of 1937–1978.

Sulfa drugs were a 20th century miracle for the treatment of bacterial and fungal infections. The first sulfa drug, Protonsil, showed no effect in vitro with bacterial assays but was extremely effective in vivo. It was later discovered that Protonsil is metabolized to sulfanilamide in vivo and the science of the bioactivation of drugs was revealed. The discovery of sulfanilamide was heralded as a major event in combating bacterial diseases. However, for a drug to be effective there needed to be an equally effective delivery system. Sulfanilamide is highly insoluble in an aqueous solution. Originally prepared as an elixir in ethanol, chemists discovered that the drug was more soluble in diethylene glycol. Therefore, the latter solvent replaced it, and a sweet syrup was added to make it more palatable to children. The new preparation was labeled an "elixir." Many patients, most of whom were children, died of acute kidney failure resulting from metabolism of the glycol to oxalic acid and glycolic acid. The drug and its metabolites crystallized in the kidney tubules, leading to renal failure (Wax, 1994). This tragedy led to the passage of the 1938 Food, Drug, and Cosmetic (FD&C) Act, also known as the Copeland Bill, named for Senator Royal S. Copeland. It contained provisions for both misbranding and adulteration. A cosmetic was deemed to be adulterated if it "contains any poisonous or deleterious substance that may render it injurious to users under customary conditions of use." The misbranding provisions prohibited labeling that is "false or misleading in any particular." The law also required that a package's ingredients and their amounts, as well as the name and address of the manufacturer, packer, or distributor, be clearly displayed on the label. To enforce the statute, the FDA was given search, seizure, and prosecutorial powers.

The sulfanilamide disaster played a critical role in the development of toxicology and inspired the research of Eugene Maximillian Geiling in the Pharmacology Department of the University of Chicago that elucidated the mechanism of toxicity of the sulfanilamide elixir (diethylene glycol). These studies began at the heart of the investigations in the late 1930s (Geiling et al., 1938). Studies of the glycols were simultaneously carried out at the FDA by a group led by Arnold Lehman, another legendary modern toxicologist.

Frances Oldham Kelsey was a research assistant in Geiling's lab at the University of Chicago during the sulfanilamide investigations and was responsible for conducting the animal toxicity testing with sulfanilamide. She earned a PhD from the University of Chicago in 1938 and graduated from Chicago's medical school in 1950. She started working at the FDA in 1960 where she was tasked with reviewing new drug applications for U.S. approval. Among her first assignments was a new drug thalidomide (Kevadon), an anti-nausea medication, also used to alleviate morning sickness in pregnant women, recently licensed by the William S. Merrell drug company based in Cincinnati, Ohio. The company had already distributed the drug to over 1200 U.S. doctors with the expectation that it would be approved quickly. Drugs could go on the market 60 days after the manufacturer filed an application with the FDA. It was often the practice of pharmaceutical companies to supply doctors with the new drugs and they were encouraged to test them on patients. Kelsey held up the application and asked Merrell for more information regarding its safety. By 1961 it became clear that thalidomide posed a serious safety risk. Infant deaths and deformities were occurring at an alarming rate across Europe and the German manufacturer began pulling the drug from the market in late 1961. By 1962 the application for approval in the United States was withdrawn completely. Though never licensed in the United States, physicians distributed the drug as samples to patients. The government estimated that more than 2 million tablets were distributed to around 20,000 patients in the United States and by late 1962 there were at least 17 babies with thalidomide-related defects. Worldwide, there were more than 10,000 babies born with thalidomide-related defects and countless pregnancies that ended in miscarriage (the exact number is unknown). The tragedy could have been far worse in the United States if not for the efforts of Frances Kelsey. The thalidomide tragedy led to the 1962 Kefauver-Harris Amendments to the FDA signed by the then President John F. Kennedy. With these amendments, the FDA was given the authority to require proof of efficacy (rather than just safety) before a new drug could gain approval. The amendments created the groundwork for the multi-phased approval process involving clinical trials, which is still very much in use today. Interestingly, under strict controls, in recent years thalidomide has been reintroduced as a treatment for certain symptoms of leprosy.

Even with the current laws in place, occasionally a drug must be highly regulated, recalled, or removed from the open market for reasons such as toxicity, impurities, lack of efficacy, or abuse potential. Clinical trials are conducted on populations significantly smaller than those eventually using the drug. Side effects not detected prior to approval often become apparent in the larger population. All other factors being equal, many effects are harder to detect in a small sample size. Increasing the sample size enhances the statistical power of a test which is the situation after approval when the drug is taken by many more people. Although drugs are often voluntarily removed from the market, there are cases where the FDA orders a drug to be recalled or removed. Mylotarg (gemtuzumab ozogamicin), for example, was approved under an accelerated approval process in 2000 for the treatment of acute myelogenous leukemia. In 2010 the drug was voluntarily withdrawn from the market by its manufacturer Pfizer. A phase 3 comparative controlled clinical trial demonstrated an increase in mortality. Additionally, the drug was not considered to be more effective over conventional cancer therapies available at the time. Vioxx (rofecoxib) was one of the largest worldwide (by Merck) recalls ever. This nonsteroidal, anti-inflammatory medication for arthritis was responsible for perhaps over 27,000 heart attacks and cardiac deaths. These effects did not emerge in the original clinical trials but subsequent trials confirmed the danger.

From around 1938 to 1971, millions of pregnant women were prescribed diethylstilbestrol (DES) as a hormone-replacement therapy and to prevent miscarriages and premature births. Research during the 1950s showed it was not effective. Before long it was discovered that DES caused a rare vaginal cancer (clear cell adenocarcinoma) in girls and young women who had been exposed to DES in the womb (Herbst et al., 1971). It was recalled from the market in 1971.

In some cases, a drug may be removed from the market temporarily to protect consumers. In 1982, there were several deaths eventually linked to Tylenol brand acetaminophen capsules. The capsules were laced with potassium cyanide (Wolnik et al., 1984). Several copycat crimes followed this incident; most notably, the conviction of Stella Nickell in 1987. Stella Nickell laced Excedrin capsules with cyanide, killing both her husband and a woman who

purchased the tampered product. Crimes such as these made clear the need for tamper-evident packaging and led to the passage of the Federal Anti-Tampering Act of 1983. Tamper-evident packaging created visual evidence for the consumer that a product was opened or damaged prior to purchase. The new packaging didn't provide 100% protection against tampering but made it much more difficult to tamper.

The FDA is routinely scrutinized by Congress, the public, drug companies, and consumer advocacy groups. Amendments and other changes are issued as the need arises according to the changing landscape of drug use, discovery, and development.

Among the latest of these changes is the process by which the FDA plans to review applications for new drugs in the future. The FDA implemented an initiative to harmonize the review and approval process for new drugs with the SEND initiative in 2016. SEND stands for the Standard for Exchange of Nonclinical Data and is an implementation of the Clinical Data Interchange Standards Consortium (CDISC) Standard Data Tabulation Model (SDTM) for nonclinical studies. The primary purpose of SEND is to present nonclinical data consistently regardless of the source of the data.

Pesticides Research and Chemical Warfare: A Surprising Alliance

Naturally derived pesticides have been used to protect crops for thousands of years. The first recorded use of insecticides took place some 4500 years ago with the Sumerians who dusted elemental sulfur on their crops. Three thousand two hundred years ago, the Chinese used mercury and arsenic compounds to control body lice (Unsworth, 2010). Synthetic pesticide development and use is a product of the 20th century. The histories of synthetic pesticide use and chemical warfare agents go at least partially hand in hand. Their research and development was widespread throughout the United States and Europe during the early 20th century. Many of the chemical warfare agents manufactured during World War I and II were discovered while conducting pesticide research. The chemicals under investigation were typically noxious chlorine derivatives and were discovered to be mildly to extremely toxic to humans. Not surprisingly, the peacetime attention to pesticide research was diverted to weaponizing many of these fortuitous discoveries during wartime. The effort behind the wartime manufacture of these agents was immense and after the war there was a surplus of what may arguably be considered the deadliest chemicals ever invented. The post-war effort was primarily geared toward disposal of these agents, although many were merely transferred and stockpiled in various countries outside of Germany. From 1946 through 1948 large amounts of various chemical weapons confiscated during World War II were dumped into the Baltic Sea after the war in a military campaign known as "Operation Davy Jones' Locker" (Kaffka, 1995). These materials continue to contaminate the waters and poison fishermen and wildlife as they are slowly released from their containers. The containers were not suitable for long-term storage and degraded over time.

Germany was responsible for much of the large-scale production of pesticides and warfare gases used in the early to mid-1900s. Fritz Haber, a German scientist, sought a way to capture nitrogen in the air for use in large-scale fertilizer production. His success, with further contributions from Carl Bosch, at nitrogen fixation (the Haber-Bosch process), garnered him the Nobel Prize in 1918. The Haber-Bosch process was instrumental in the manufacture of nitrogen-based explosives for the German Army during World War I (Hager, 2009). Some argue that the Germans would have run out poisonous gases if not for Fritz Haber and Carl Bosch. Bosch also researched the weaponization of toxic substances such as chlorine, phosgene, and mustard gas, leading to the largest deployment of chemical weapons in modern history. During World War I, the Germans launched a chemical attack using chlorine gas in Ypres, Belgium in 1915. Phosgene, which is now used in the manufacture of pesticides and plastics, was employed extensively by the Germans during World War I and accounted for nearly 85% of all gas-related fatalities during that war (Marrs et al., 2007). Tabun was the first nerve agent to be synthesized in 1937 by the IG Farben scientist Gerhard Schrader during his research to discover new organophosphate insecticides. The human toxicity of tabun was realized by accident during its development in 1935. Tabun causes acetylcholinesterase inhibition in the peripheral and central nervous systems. The symptoms that result include trembling, convulsions, and respiratory paralysis. During World War II, tabun was manufactured as a part of the Grün 3 program in Brzeg Dolny, Poland in 1942. The plant was seized by the Soviet Army and moved to Russia. The production and stockpiling of chemical warfare agents continued throughout World War II. In the 1930s Willy Lange (a German biochemist) and Gerhard Schrader also discovered organophosphate cholinesterase inhibitors including sarin, soman, cyclosarin, and other less potent organophosphate insecticides. This class of chemicals was destined to become a driving force in the study of