## SUBJECT
**FORENSIC SCIENCE**

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1. Learning Outcomes

After studying this module, you shall be able to know that –

- What is Forensic Toxicology?
- Sub-divisions of Forensic Toxicology.
- Concept of Forensic Toxicology and its Significance.
- Development and Advancement of Forensic Toxicology.

2. Introduction: Toxicology

The word “Toxicology” is came from the Greek word “toxicon” which was used as a poisonous substance in arrowheads. Conventionally, the toxicology may be defined as the science representing the character, source, knowledge, lethal dose, fatal effect, analysis of poisons and their curative measures. More precisely, Toxicology can be said as the study of antagonistic effects of chemical or physical agents on living organisms. A “toxicologist” is competent to examine and lead into the nature of those effects on human, animal, and environmental health. Toxicological study examines the cellular, biochemical, and molecular mechanisms of action as well as functional effects such as neurobehavioral and immunological, and assesses the likelihood of their event.

The diversity of chemicals and that of their prospective adverse effects in the environment make toxicology a comprehensive science, which often demands specialization in a specific range of toxicology. Our society’s dependency on chemicals and the requirement to assess impending hazards have made toxicologists an increasingly important part of the decision-making processes. The existing description would be somewhat altered but not necessarily be incorrect as it regards with the study and analysis of Physical and Chemical properties of Poisons. Where the Physical properties means the appearance e.g., colour, state, Melting Point, Boiling Point, etc; the Chemical properties has an important characteristic in which the fatal dose, toxicity, action upon the body are studied including its metabolism. The added labour in the field of Toxicology is the detection of any poison inside the body, its Quantitative and Qualitative estimation and interpretation of results of the respective toxicological analysis. Poison may be regarded as any substance which, when taken in sufficient quantity, will cause ill health or death. The adage in this definition is “sufficient quantity”.
3. History of Toxicology

The earlier Indian text *Rig Veda* (12th century BC) also describes several plant poisons. However, Toxicology dates back to the earliest humans, who used plant extracts and animal venoms for warfare, hunting, and assassination. Elucidating the mechanisms of the toxicity of venoms continues today in the field of toxicology. The knowledge of these poisons must have pre-existed detailed history. According to a presumption, prehistoric humans characterized some plants as hazardous and others as safe. The same is possibly true for the classification of snakes and other animals.

The ancient book *Ebers Papyrus* contains information pertaining to many known poisons, including hemlock, aconite, opium, and metals such as arsenic, lead, copper, and antimony. Among mineral poisons, one of the earliest known elements was lead which was discovered as early as 3500 BC. Apart from its extensive use in plumbing, lead was also employed in the production of vessels and containers, which led to pervasive chronic health problems. There is also a suggestion that plants containing substances similar to digitalis and belladonna alkaloids were known to them. Hippocrates kept various poisons and clinical toxicology principles relating to bioavailability in remedy and over dosage, whereas the Book of Job states about of poison arrows. In the literature of ancient Greece, there are numerous references to poisons and their use. Some explanations of Homer have Odysseus attaining toxins for his arrows.

*Theophrastus*, a student of Aristotle, comprised many references to poisonous plants in *de historia plantarum*. *Dioscorides*, a Greek physician in the court of the Roman emperor Nero, did the first effort at a classification of poisons, which was supplemented by descriptions and diagrams. His classification into plant, animal, and mineral poisons not only remained a standard for 16 centuries but also is still a convenient classification. *Dioscorides* also experimented in remedy, identifying the use of emetics in poisoning and the use of caustic agents and cupping glasses in snakebite. Poisoning with plant and animal toxins was quite common. Perhaps the best known recipient of poison used as a state method of execution was Socrates (470–399 BC), whose cup of hemlock extract was apparently estimated to be the proper dose.

Although, Organic chemistry was in its nascent stage till 1800, but by 1825 war gases like Phosgene (COCl₂) and Mustard Gas (bis [β-chloroethyl] sulphide) had been synthesized successfully.

These two agents, along with Chlorine gas, were used by the German forces in World War I as chemical warfare agents. They were stored throughout World War II, and used by Iraq in the Iran–Iraq War in the 1980s (Marine Corps History). By 1880 over 10,000 organic compounds had been manufactured including chloroform, carbon tetrachloride, diethyl ether, and carbonic acid, and petroleum and coal gasification by-products were used in trade. Experimental toxicology accompanied the growth of organic chemistry and developed rapidly during the 19th century.
4. Classification of Toxicology

The field of toxicology can be further divided into the following sub-disciplines:

1) Environmental Toxicology:

Environmental Toxicology is concerned with the study of chemicals that contaminate food, water, soil, or the atmosphere. It also deals with toxic substances that enter waters bodies such as rivers, lakes, streams, and oceans. This category of Toxicology describes the effect of exposure of toxic substances on living organism like plants, animals, and humans.

2) Occupational Toxicology:

Occupational Toxicology or Industrial Toxicology is concerned with health effects from exposure to chemicals in the workplace. This field grew out of a need to protect workers from toxic substances and to make their work environment safe. Occupational diseases caused by industrial chemicals account for an estimated 50,000 to 70,000 deaths, and 350,000 new cases of illness each year in the United States. According to WHO, there are 100 million occupational injuries causing 0.1 million deaths in the world. It is also estimated that 17 million occupational non-fatal injuries (17% of the world) and 45,000 fatal injuries (45% of the total deaths due to occupational injuries in world) occur each year in India. Out of 11 million cases of occupational diseases in the world 1.9 million cases (17%) are contributed by India and out of 0.7 million deaths in the world 0.12 (17%) is contributed by India.

3) Regulatory Toxicology:

Regulatory Toxicology gathers and evaluates existing toxicological information to establish concentration-based standards of “safe” exposure. There are serious differences between the purposes and procedures of science and regulatory policy. Science pursues to recognize natural phenomena through unprejudiced, pragmatic, and impartial practices, and is careful, incremental, and evidence-based as well.

4) Food Toxicology:

Food Toxicology is involved in delivering a nontoxic and edible supply of food to the consumer. During processing, a number of substances may be added to the food to make it taste, smell, or look better. Fats, oils, sugars, starches and other substances may be added to change the texture and taste of food. All of these additives are studied to conclude if and at what amount, they may produce adverse effects. A second area of interest includes food allergies. For example, many people have trouble digesting milk, and are lactose intolerant. Furthermore, toxic substances like pesticides may be applied to a food crop in the field, while lead, arsenic, and cadmium are naturally present in soil and water, and may be absorbed by plants. Toxicologists must determine the tolerable daily intake level for those substances.
5) Clinical Toxicology:

Clinical Toxicology is concerned with diseases and illnesses associated with short term or long term exposure to toxic chemicals. Clinical toxicologists include emergency room physicians who must be familiar with the symptoms related with exposure to an extensive variety of toxic substances in order to manage the appropriate treatment.

6) Descriptive Toxicology:

Descriptive Toxicology is concerned with collecting toxicological statistics from animal experimentation. These types of experiments are used to establish how much of a chemical would cause illness or even death. A descriptive toxicologist is concerned directly with toxicity testing, which provides information for safety evaluation and regulatory requirements. The appropriate toxicity tests in cell culture systems or experimental animals are designed to yield information to assess risks posed to humans and the environment from exposure to specific chemicals. The concern may be limited to effects on humans, as in the case of drugs and food additives.

Toxicologists in the chemical industry, however, must be concerned not only with the risk posed by a chemicals like insecticides, herbicides, solvents, etc., to humans but also with potential effects on fish, birds, and plants, as well as other factors that might disturb the balance of the ecosystem. Descriptive toxicology is of course not separated from mechanistic studies, as such studies provide important clues to a chemical’s action mechanism, and consequently contribute to the development of mechanistic toxicology through hypothesis generation. Such studies are also a key component of risk calculations that are used by regulatory toxicologists.

7) Analytical Toxicology:

Analytical toxicology identifies the toxicant through analysis of body fluids, stomach content, excrement, or skin. It also comprises the application of the tools of analytical chemistry to the qualitative and/or quantitative assessment of chemicals that may exert effects on living organisms. Generally, the chemical that is to be measured is a xenobiotic that may have been altered or transformed by metabolic actions of the organism. Frequently, the specimen that is to be analyzed consists of a matrix composed of body fluids or solid tissues removed from the organism. Both the identity of the analyte and the complexity of the matrix can present difficulties to an analytical toxicologist.
8) Mechanistic Toxicology:

Mechanistic Toxicology makes observations on how toxic substances cause their effects. The effects of exposure can depend on a number of factors, including the size of the molecule, the exact tissue type or affected cellular components, whether the substance is easily dissolved in water or fatty tissues, all of which are important when trying to determine the way a toxic substance causes harm, and whether effects seen in animals can be expected in humans.

9) Forensic Toxicology:

Forensic Toxicology is used to help establish cause and effect relationships between exposure to a drug or chemical and the toxic or lethal effects that result from that exposure. Forensic Toxicology talks about to the application of conventional toxicology for the purposes of the Criminal Investigation to assist legal administration. It can be considered as a hybrid of Analytical Chemistry and Fundamental Toxicology with advanced Forensic Medicine. Forensic toxicology is also primarily concerned with the medico-legal characteristics of the detrimental effects of chemicals on human and animals. The expertise of forensic toxicologists is primarily utilized in establishing the cause of death and interpreting its circumstances in post-mortem investigation.

5. Concept of Forensic Toxicology and its significance

For a Forensic Toxicologist, the mechanism of toxicity often provides perception as to how a chemical or physical agent can cause death or induce incapacitation. If the mechanism of toxicity is understood, descriptive toxicology becomes useful in predicting the toxic effects of related chemicals. Toxicity is the fundamental capacity of a chemical agent to affect an organism harmfully. These agents are rather termed as Xenobiotics i.e., foreign substance. Xenobiotics include drugs, industrial chemicals, naturally occurring poisons and environmental pollutants. Risk is the possibility of a specific adverse effect to occur. It is often expressed as the percentage of cases in a given population and during a specific time period. A risk estimate can be based upon actual cases or an estimation of future cases, based upon hypothetical calculations. Toxicity rating and toxicity classification can be used for regulatory purposes. Toxicity rating is an arbitrary grading of doses or exposure levels causing toxic effects. These grading may be “super toxic,” “highly toxic,” “moderately toxic” and so on. The most common ratings concern acute toxicity. Toxicity classification concerns the grouping of chemicals into general categories according to their most important toxic effect. Such categories can include allergenic, neurotoxic, and carcinogenic, etc. This classification can be of administrative value as a warning and as information.
Information from the toxicological sciences, gained by experience or research, has a growing influence on our personal lives as well as on human and environmental health across the globe. Knowledge about the toxicological effects of a compound affects consumer products, drugs, manufacturing processes, waste clean-up, regulatory action, civil disputes, and broad policy decisions. The expanding influence of toxicology on social issues is accompanied by the responsibility to be increasingly sensitive to the ethical, legal, and social implications of toxicological research and testing.

Poisoning has become one of the commonest medical emergencies throughout the world because thousands of pharmacological and chemical agents are commonly used and their numbers are increasing every year. The incidences of poisoning and substance abuse have been gradually rising in India over the last few decades. Accurate diagnosis of poisoning is essential, both in the living as well as in the dead, for therapeutic and medicolegal purposes respectively. Poisons are generally detected in body fluids such as urine, blood, or gastric lavage during life, while they are detected in the contents of stomach, bowel and the viscera, besides urine and vomitus, after death.

In a cadaver, one of the better samples to complement blood is vitreous humor, for the reason that it is less likely to degrade quickly, and is equitably easy to collect at time of autopsy. Forensic toxicological analysis has traditionally focused on the use of body fluids including blood along with certain organs in examinations of deaths due to intoxication. However, in some circumstances, putrefaction and contamination make proper sampling from tissues difficult, such as in exhumation cases. In these cases, bone marrow might be useful as an alternative specimen since it is a potential depot for drugs.

Determination of the toxicological potential of these newly created chemicals became the underpinning of the science of toxicology as it is practiced today. The science of toxicology has expanded to include a wide range of interests, including the evaluation of the risks involved in the use of pharmaceuticals, pesticides, and food additives, as well as studies of occupational poisoning, exposure to environmental pollution, the effects of radiation, and, regrettfully, biological and chemical warfare. Nevertheless, it is the forensic toxicologist who has held the title of toxicologist for the longest period of time. The forensic toxicologist is concerned primarily with the detection and estimation of poisons in tissues and body fluids obtained at autopsy or, occasionally, in blood, urine, or gastric material obtained from a living person. Once the analysis is completed, the forensic toxicologist then interprets the results as to the physiological and/or behavioural effects of the poison upon the person from whom the sample was obtained.

All substances can act as poison if they are used in an inappropriate way. The inappropriate way may contain their dosage, state of appearance and mode of administration. For example, normal sugar which is taken in our daily consumption may act as a trigger to diabetic attack if taken in overdose. Similarly, the normal oxygen we inhale may kill a person if even a bubble is injected intravenously. Swiss Alchemist and Physician Paracelsus as well as Italian physician Ramazzini also noted the toxicity of smoke and soot.
6. Development and Advances of Forensic Toxicology

Criminal convictions related with homicidal poisoning until the 17th century was solely based on circumstantial evidences rather than the identification of the definite toxicant which purportedly killed or harmed the victim. In 1781, Joseph Plenic stated that the identification and detection of poison in the organs of the deceased was the only true sign of poisoning.

Later in 1813, a Spaniard, Mathieiv Joseph Bonaventure Orfila (Mathieu Orfila), who was working at the University of Paris in the early nineteenth century, published the first complete work on the subject of poisons and legal medicine. His treatise *Traite des Poisons* published in 1814 laid the foundations of forensic toxicology. He clearly identified Toxicology as a separate science which gained him the title “Father of Modern Toxicology”.

By 1836, James M. Marsh developed a test for the detection of Arsenic in body tissue which is still used to identify the presence of Arsenic in Homicidal Poisoning cases and known as Marsh Test. Some years later one Ernst Wilhelm Heinrich Gutzeit discovered a method to quantitate Arsenic in tissues. In this process arsenic compounds are reduced by hydrogen which is produced when Zinc and Sulphuric Acid reacts. The hydrogen then reduces the arsenic compounds to Arsine, which is exposed to paper that has been treated with Mercuric Chloride solution. This produces a colour range from yellow to brown depending on the arsenic concentration. This process is still in use and known as the Gutzeit Test. By 1918, the Medical Examiner’s Office and Toxicology Laboratory was established in New York. The Chief Forensic Toxicologist was Alexander O. Gettler who is considered the Father of American Toxicology.

Following the end of World War II, the era of instrumentation began. Instrumentation enabled more accurate quantification of toxic substances in tissue and multiple testing by using automated equipment. Combining the automated equipment with computers allowed multiple analyses, calculations, and printing and storage of the results. The first successful isolation of an alkaloid poison was performed in 1850 by a Belgian chemist, Jean Servials Stas, using a solution of Acetic Acid in Ethyl Alcohol to extract Nicotine from the tissues of the murdered Gustave Fougnie. Improved by the German chemist, Friedrich Otto, the Stas-Otto method was quickly applied to isolation of numerous alkaloid poisons, including colchicine, conin, morphine, narcotine, and strychnine; the method is still used today.

In India, the toxicological analysis of Viscera is done in the Toxicology Division of Forensic Science Laboratories or Department of Forensic Medicine of Medical Colleges. Generally the understanding is that whenever a death case is reported, the body is autopsied under the supervision of a Senior Expert or the Head of the Department of the Forensic Medicine Department and the Viscera is preserved in such a manner that it can be safely transported to the concerned Forensic Science Laboratory for the further analysis.
In the Forensic Science Laboratory, after the approval of Director, authorised expert commences the requisite examinations to detect poisonous substance and accordingly prepare its report. Report provided by forensic toxicology personnel and expert consultants may ultimately be introduced as evidence in a court of law. These reporting individuals may be asked to interpret and substantiate their findings and any associated opinions. It is therefore necessary that the forensic toxicologist be thoroughly knowledgeable or familiar with legal practices and be professionally comfortable in a courtroom environment.

As the time passed and the modern innovative techniques crossed through the boundaries of inter related science, the instrumental intervention significantly enhanced the field of toxicological analysis. Latest instruments with remarkable preciseness facilitated deciphering the crucial dilemmas of the in-depth toxicological examination. It is amply cleared that the variety of potential adverse effects and the diversity of chemicals present in our environment contribute to make toxicology a very broad field of science. The analytical techniques employed by forensic toxicologists have continued to expand in complexity and improve in reliability and sensitivity. Many new analytical tools have been applied to toxicological problems in almost all areas of the field, and the technology continues to open new areas of research.

The toxicology and drug identification section of the Forensic Laboratory utilizes forensic chemistry and modern instrumental techniques to isolate, identify, and often quantify alcohol, drugs, poisons, and other toxic materials. The material submitted for analysis may consist of bulk quantities of illicit drugs such as Marijuana, Heroin, LSD, or Cocaine which must be accurately weighed, analysed, and often quantitated to establish the degree of a criminal drug charge. Other liquid, solid, or gaseous materials may be submitted for analysis to determine the presence any toxic or poisonous content. Many crime laboratories analyse blood for alcohol and/or drug content in cases of driving while impaired or intoxicated, as well as perform alcohol, drug, and toxic substance analysis on body tissues and fluids for purposes of death investigation in conjunction with the medical examiner’s office. Drugs detected may be either of a prescribed or illicit nature. Some commonly encountered toxic substances are carbon monoxide, cyanide, insecticides, and heavy metals. The quantitative blood and tissue levels of these substances, as well as their metabolites, may reveal therapeutic, toxic, or lethal levels present in the body and help determine the role of the substance in the death of the individual. Occasionally, the determination of the absence of a prescribed medication, such as Anticonvulsive Drugs in victims known to have a Seizure Disorder, may help to explain their behaviour or circumstances of a death. The procedures used in a typical toxicology section of a laboratory may include extraction and purification techniques prior to analysis.
Preliminary examination of materials may include chemical spot tests which by colour production may indicate the existence of a type of drug or poison. More sophisticated techniques include Ultraviolet Absorption (UV), Infrared Absorption (IR), Radioimmunoassay (RIA), Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), and Gas Chromatography (GC). The application of Mass Spectroscopy (MS) is now commonly employed for identification of drug and toxic compounds.

7. Summary

1. The word “Toxicology” is derived from the Greek word “toxicon” which was used as a poisonous substance in arrowheads.
2. Poison may be regarded as any substance which, when taken in sufficient quantity, will cause ill health or death. The adage in this definition is “sufficient quantity”.
3. The field of toxicology can be further divided into the various sub-disciplines, such as Environmental, Occupational, Regulatory, Food, Clinical, Descriptive, Analytical, Mechanistic and Forensic Toxicology.
4. Toxicity is the fundamental capacity of a chemical agent to affect an organism harmfully. These agents are rather termed as Xenobiotics i.e., foreign substance.
5. Toxicity classification concerns the grouping of chemicals into general categories according to their most important toxic effect. Such categories can include allergenic, neurotoxic, and carcinogenic, etc.
6. The toxicology and drug identification section of the Forensic Laboratory utilizes forensic chemistry and modern instrumental techniques to isolate, identify, and often quantify alcohol, drugs, poisons, and other toxic materials.