The science of toxicology

Introduction to Toxicology:

The science of Toxicology consists of the study of biology, chemistry, and medicine, that is concerned with study of harmful; effects of chemicals on living organisms. It also studies the harmful effects of the chemical, biological and the physical agents in biological systems that establish the extent of damage in living organisms. The relationship between the given dose and its effects on the exposed organism is of very high significance in toxicology. Variables that influence chemical toxicity, includes the given dosage, the probable route of exposure, species, age, sex and environment.

A toxicologist is a scientist or medical personal who specializes in the study and observation of symptoms, function and mechanism, treatments and detection of venoms and toxins; especially in case of poisoning. To work as toxicologist one should get a degree in toxicology or a related field like biochemistry and the life sciences.

The main branches of toxicology are:

Forensic toxicology:

It is the use of toxicology and other disciplines such as pharmacology, chemistry such as analytical chemistry and clinical chemistry to aid medical or legal investigation of death due to poisoning, and drug use. The chief concern for forensic toxicology is not always the legal outcome of the toxicological investigation or the technology used, but rather the obtaining and interpreting of the evidence and results. A toxicological analysis now can be done to various kinds of samples.

A forensic toxicologist must minutely consider the context of an investigation, particularly any physical symptoms that are recorded, and any evidences collected at scene of the crime that helps in narrowing the search, such as any available chemicals powders and/or trace residue. Armed with this information and samples with which to work, the toxic substances that are present there, its concentrations, the probable chemicals effects on the person, all of these information are determined by the forensic toxicologist.

In vitro toxicity:

It is the scientific analysis of the effects of toxic chemical substances on cell cultured bacteria or mammalian cells. These methods are used primarily to identify dangerous chemicals, to verify the lack of certain toxic properties in the early stages of development of potentially useful new substances like therapeutic drugs, agro chemicals, food colours and additives and other useful substances.

In vitro assays for xenobiotic toxicity are carefully considered by major government organizatios (e. g. EPA, NTP, FDA), to better assess human risks. There are major activities in using in vitro systems to advance understanding of toxicant activities, and the use of human cells, tissues and organs to define human-specific toxic effects.

Environmental toxicology:

It is a multidisciplinary field of science concerned with study of the harmful effects of various chemical agents, biological agents and physical agents on living organisms. it is a sub discipline of environmental toxicology that is concerned with studying the harmful effects of toxicants, at the general population and ecosystem levels.

Medical toxicology:

It is a medical subfield focusing on the diagnosis of health problems, their management and prevention of adverse health effects such as poisoning and other complications from medications, occupational toxicants, toxicants in the environment, and/or various other biological agents. Medical toxicologists personal are involved in the assessment and treatment for poisoning, the harmful drug reaction, overdoses and substance abuse.

Medical toxicology practitioners are physicians, whose primary specialization is generally in emergency medicine, occupational medicine or pediatrics.

Ecotoxicology:

It is the study of the effects of toxic chemicals on the biological organisms, at the population, community and at the ecosystem levels. Study of Ecotoxicology is a multidisciplinary field, which combines toxicology and ecology.

The ultimate aim of this approach is to be able to predict the effects of pollution so that efficient and effective action to prevent or remediate any adverse effect can be identified. In the ecosystems that are already affected by pollution, Eco toxicological studies can inform as to the best method for action to restore the ecosystem efficiently and effectively.

Ecotoxicology differs from science of environmental toxicology in that it combines the effects of stressors across all the levels of biological organizations i. e. from the molecular to whole communities and ecosystems, whereas science of environmental toxicology focuses upon the effects at level of the individual and below.

Entomotoxicology:

It is the analysis of toxins in arthropods that feed on carrion. Using arthropods in corpse or at crime scene, investigators can correctly determine whether toxins or poisons were present in a body at the exact time of death. This technique is a major advancement in forensics. Before, such determinations were impossible in the case of the severely decomposed bodies, which were devoid of intoxicated tissue and body fluids. Ongoing researches into the effects of toxins on arthropod and their development has also allowed better estimations of the postmortem intervals.

Forensic entomology is the application and also the study of insects and other arthropod biology to criminal matters. It also involves application of study of arthropods, such as insects, the arachnids, the centipedes, and millipedes, crustaceans to the criminal or legal proceedings. It is mainly associated with death investigations; however, it may also be used to detect drugs, poisons and determine the location of an incident, and also find the presence and time of when the wounds were caused. Forensic entomology can thus be further broken under three subparts: urban, stored-product and lastly medico-legal/medico-criminal entomology.

Toxinology:

It is the specialized field of science that deals mainly with the animals, plants, and microbial toxins. It has been defined as “ the scientific discipline dealing with microbial toxins, plant toxins, and animal venoms”. This involves more than just the chemistry and mode of action of toxins. It deals with the working of venom, the poison-producing organisms, also the structure and functions of the venom glands, use of the venom or poison and

also the ecological role of these compounds. Toxinology has also been further defined as “ the science of toxic substances produced by or stored in living organisms, their properties, and their biological importance for the organisms involved”.

Clinical toxinology:

Within toxinology there is also a subgroup, i. e. clinical toxinologists, who studies the medical effects in humans, exposure to the toxins, also in animal venoms or in plant poisons. This includes problems such as venom from snakebite, currently considered to affect more than 2. 5 million patients each year, with over more than 100, 000 deaths.

Clinical toxinology does not have specialist status yet within the field of medicinal study, unlike other fields such as surgery and radiology. However, training courses in clinical toxinology exists.

Sample Preparation:

Sample preparation is often the first step in an analysis; the result of this step can affect the rest of the analytical process. To get accurate results, a sample should be representative, it should be reproducible, homogenous, and must be suitable for column injection or other assay.

The main steps in sample preparation are:

1. Sample Identification
2. Sample reagent and standard pipetting
3. Sample extraction
4. Output to analyzer format

Preparative Steps:

- Removal of Soluble Protein

– precipitation

– filtration

- Extraction

– single step liquid-liquid extraction

– Multiple step liquid-liquid extraction (“ back-extraction”)

– solid phase extraction

- Chemical Modification

– derivatization for increase in volatility of sample

– chemical hydrolysis of glucuronide enzyme

- Concentration

– evaporation

- Cell lysis or tissue homogenation

Sample Characterization:

There are many chromatographic assays (GC, GC/MS, HPLC, TLC, LC/MS/MS,), that are used for characterization and toxicological analysis of sample.

To understand them, it is best to break them down into their modular components/steps:

1. Sample preparation
2. Separation (the actual chromatography)
3. Detection (UV/Vis spectrometry, Fluorescence spectrometry, Mass spectrometry).

Chromatographic Components:

1. Sample “ loading”
2. The “ mobile phase” during separation.
3. The “ stationary phase” during separation.

Separation of individual molecules in the sample components is always based on their relative affinity for the mobile phase versus the stationary phases.

Because some of the molecules have higher affinity for the stationary phase, they will pass through column slower than the others and, therefore, will be separated.

Separation of the different Molecules by Chromatography:

- After the injection, all molecules start out overlapping.
- Due to the varying relative affinity for the stationary phase versus the mobile phases, individual molecules thus begin to separate
- As the different molecules then elute off of the column, they are then detected as resolved “ peaks”.

Relative Retention Times:

- During the separation, the absolute rates/times for movement of the molecules are not always reproducible. For example, the columns can

get dirty, thus decreasing the amount of stationary phase that is available for the interaction with molecules.

- This can be compared to shortening the length of the column. However, it affect the rate and all molecules in the same way.
- Therefore, their relative rates/times are highly reproducible. The “ relative retention time” (RRT) is defined as the detection time for a individual peak divided by the detection time for a known internal standard.
- RRTs are characteristic and reproducible identifiers of individual molecules.

Quantification of Drug Concentrations:

- Peak “ area” generally correlates with the amount of drug that is loaded onto a column and on the original drug concentration. But, there can be sample-to-sample variations due to the extraction efficiency, the loading volumes, or the detection efficiency, etc.
- Again, the internal standard is utilized to correct for variations.–Similar to the relative retention time, relative peak intensity is defined and related to drug concentration.
- Unlike the relative retention time, the given variation in the peak area is not always similar for all the molecules. Thus, the internal standard is chosen to be chemically similar to the analyte of interest to best correct for variations. However, adequate similarity is not easy to predict or establish.

Protocol for Quantification of Analyte Concentration Based Upon a Calibration Curve:

- A known quantity of an internal standard is first added to every sample (including controls and calibrators) before any other preparative step.
- All samples are then prepared through the identical preparative steps, separated by a chromatographic method and quantitatively detected.
- The relative peak intensities are measured for a series of calibrators with a fixed amount of internal standard and varying amounts of a known analyte.
- These relative peak intensities are fit to an equation, generally linear, to define a calibration curve.
- The relative peak intensities of unknown samples are then calculated and then related to the calibration curve to quantify the concentration of the analyte(drug) in the original clinical sample.

Some Characterization Techniques:

Affinity Chromatography:

Affinity chromatography is used for separating biochemical mixtures based on the highly specific interaction between conjugates such as that between antigens and antibodies, enzymes and substrates, or receptors and ligands.

Principle:

Here, the stationary phase used is typically a gel matrix, often of agarose. Generally, we use an undefined heterogeneous group of molecules in solution, like, for example, growth medium or blood serum. The molecule of interest will be having a well-defined property, and can be put to use during the affinity purification process. This process can thus be seen as a process of entrapment, with target molecule getting entrapped on solid or stationary phase and/ or medium. The molecules of mobile phase component will not

become trapped as they do not possess this property. The stationary phase is then removed from the mixture, washed and target molecule released from entrapment in process known as elution. The most common use of affinity chromatography is for the purification of recombinant proteins.

Affinity chromatography has use in number of applications, including purification from nucleic acid, and purification from blood and also protein purification from cell free extracts.

Thin-layer chromatography (TLC):

It is a chromatography technique used to separate non-volatile and stable mixtures. Thin-layer chromatography analysis is performed on sheet of various mediums, such as glass, plastic, or aluminum foil, they are then coated with a thin layer of adsorbent material, like silica gel, cellulose and also aluminum oxide. This layer is known as the stationary phase.

After the sample is applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes have different rate of ascension on the TLC plate, separation is achieved.

It can monitor the progress of a reaction, or determine the purity of substances and/or identify the compounds present in a given mixture. Some examples are: analyzing the fatty acids, detection of pesticides , herbicides and/or insecticides in food and water, analyzing ceramides, analyzing the dye composition of fibers in forensic toxicology, or identification of medicinal plants and their constituents and assaying the radiochemical purity of radiopharmaceuticals.

A number of enhancements to the original method have been made, to increase the resolution achieved with TLC, to make the different steps automatic and to allow more accurate quantitative analysis. This is called HPTLC, or “ high-performance TLC”.

Summary of Major Learning Points:

- Modular nature of chromatograpy.

– Assays are divided into three steps: *sample preparation, sample component separation* and *analyte detection* .

– The separation steps consist of sample *loading,* preparing a *mobile phase* and a *stationary phase* .

- Importance of an internal standard for

– Calculating the *relative retention times* for component separation *.*

– Calculation of the *relative peak areas* and the generation of a calibration curve for the quantification of drug concentrations in the original clinical sample.

- Analytical *specificity* provided by

– Sample preparation techniques

– Separation during chromatography (RRT)

– Method chosen for detection