Introduction to toxicology

During the past decades industrialization and agricultural development, paralleled with increased health care have changed life in various ways. Average life expectancy rose, due to better control of epidemics and infectious diseases. However, increased industrialization and agricultural development were the chief cause of pollution that had profound influences on our lives. Man, the other animals, & the plants in the modern world are increasingly being exposed to chemicals of an enormous variety. Nearly everyone is at risk of toxic exposures to hazardous substances in the ambient environment. In recent years, awareness of the problem of human & animal exposure to potentially toxic chemicals in our environment has grown. So toxicology has a very important role to play in modern society & consequently it is now growing rapidly as a new subject.

1. **What is toxicology?**

The word toxicology is derived from two Greek words; toxikon, meaning poisonous substance into which arrow heads were dipped and logos, meaning study.

Toxicology is the qualitative and quantitative study of the adverse or toxic effect of chemicals and other anthropogenic materials or xenobiotics on organisms. It also deals with food and cosmetics for public consumption both in alive or dead victims.

It is the science of poison & its scope has been enlarging. It is one of the multidisciplinary fields of science.

It has got another dimension: the social, the moral & legal aspects of exposure of populations to chemicals of unknown or uncertain hazard.

Historical aspects of toxicology – it is only recently that the study of poisons becomes truly scientific & in the past it was mainly a practical art utilized by murderers & assassins.

Poison has played an important part in human history.

In Ancient time (1500 BC) earliest collection of medical records contains many references and recipes for poisons.

Dioscorides (50 AD) a Greek physician, classify poisons as animal, plant or mineral & recognized the value of emetics Maimmonides (1135-1204 AD), wrote about poisons and their antidote.

Paracelsus (1493 AD), viewed a poison in the body would be cured by a similar poison but the dosage is very important. Paracelsus summarized his concept in the following famous phrase "All substances are poisons; there is none that is not a poison. The right dose differentiates a poison from a remedy"

Orifila (1787-1853 AD), Spanish physician who contributed to forensic toxicology by devising means of detecting poisonous substances. From then on toxicology began in a more scientific manner & began to include the study of the mechanism of action of poisons.

The 20th century- toxicology has now become much more than the use of poisons. There are marked improvements in toxicological diagnosis (that ranges from screening to confirmatory tests), & management (production of antidote for them).

2. Epidemiology

Poisoning (both accidental and intentional), is a significant contributor to mortality and morbidity. It has been estimated that 7% of all emergency room visits are the result of toxic exposures. Household cleaner, over-the-counter and prescription drugs, cosmetics, and solvents comprise the most frequent human toxic exposures. Young children and elderly are most likely to be accidentally exposed to drugs or household chemicals at home. During adolescence and young adulthood the exposures are more likely to be intentional, either through suicide attempts or experimentation with drugs or alcohol. More than 72.4% of all poison exposures occur in children and adolescents less than 17 years of age. Exposures are equally reported in males and females. However, adult men

have been reported to be more at risk of occupational exposures than adult woman. Route of entry of exposures reported was by mouth in most cases: 77% were the result of ingestion, 7.0% were transdermal, 5.9% were ophthalmic; and 5.5% were by inhalation. Site of exposure was a residence in 91.9% of all, followed by the workplace, schools and health facilities. Most poison exposures do not result in clinical toxicity. In general, nearly everyone is at risk of acute and chronic toxic exposures to hazardous substances in the ambient environment.

3. Toxicologic terms and definitions

A) Important toxicologic terms

Toxin- a poison of natural origin.

Poison (Toxicant) - a chemical that may harm or kill an organism

Toxicity – is the ability of a chemical agent to cause injury. It is a qualitative term which depends on the amount of chemical absorbed, severity of the exposure, dose & others. It can be acute (toxic event which occurs soon after acute or limited exposure), or chronic (apply to an event which occurs many weeks, months or years after exposure).

Hazard – is the likelihood that injury will occur in a given situation or setting: the conditions of use and exposure are primary considerations.

Risk – is defined as the expected frequency of the occurrence of an undesirable effect arising from exposure to a chemical or physical agent.

Acute exposure is a single exposure – or multiple exposures occurring over 1 or 2 days.

Chronic exposure is multiple exposures continuing over a longer period of time.

B) Presence of mixtures

Humans normally come in contact with several (or many) different chemicals concurrently or sequentially. The resulting biologic effect of combined exposure to several agents can be characterized as **additive, synergistic, Potentiation & antagonistic**

An additive effect occurs when the combined effect of two chemicals is equal to the sum of the effects of each agent given alone (example: 2 + 3 = 5). For example, when two organophosphate insecticides are given together, the cholinesterase inhibition is usually additive.

A synergistic effect occurs when the combined effects of two chemicals are much greater than the sum of the effects of each agent given alone (example: 2 + 2 = 20). For example, both carbon tetrachloride and ethanol are hepatotoxic compounds, but together they produce much more liver injury than the mathematical sum of their individual effects on liver at a given dose would suggest.

Potentiation occurs when one substance does not have a toxic effect on a certain organ or system but when added to another chemical makes that chemical much more toxic (example: 0 + 2 = 10). Isopropanol, for example, is not hepatotoxic, but when it is administered in addition to carbon tetrachloride, the hepatotoxicity of carbon tetrachloride is much greater than when it is given alone.

Antagonism occurs when two chemicals administered together interfere with each other's actions or one interferes with the action of the other (example: 4 + 6 = 8). Chelates with metal ions such as arsenic, mercury, and lead decreases their toxicity

4. **Basic classification of toxicology**

Toxicology is broadly divided into different classes depending on research methodology, socio-medical & organ/specific effects.

I. Based on research methodology

A. Descriptive toxicology

Descriptive toxicology deals with toxicity tests on chemicals exposed to human beings and environment as a whole.

B. Mechanistic toxicology

Mechanistic toxicology deals with the mechanism of toxic effects of chemicals on living organisms. This is important for rational treatment of the manifestations of toxicity (e.g

. organophosphate poisoning reversed by oximes), prediction of risks (e.g. organophosphate poisoning leads to accumulation of acetylcholine activate muscarinic and nicotinic receptors respiratory failure) & facilitation of search for safer drugs (e.g. Instead of organophosphates, drugs which reversibly bind to cholinesterase would be preferable in therapeutics)

C. Regulatory toxicology

Regulatory toxicology studies whether the chemical substances has low risk to be used in living systems E .g - Food and drug administration regulates drugs, food, cosmetics medical devices &supplies.

Environmental protection agency regulates pesticides, toxic chemicals, hazardous wastes and toxic pollutants.

Occupational safety and health administration regulates the safe conditions for employees.

Drug administration & control authority (DACA) - regulates drugs, cosmetics and medical devices &supplies.

D. Predictive toxicology

Predictive toxicology studies about the potential and actual risks of chemicals /drugs. This is important for licensing a new drug/ chemical for use.

II. **Based on specific socio-medical issues**

A) Occupational toxicology

Occupational toxicology Deals with chemical found in the workplace E.g. – Industrial workers may be exposed to these agents during the synthesis, manufacturing or packaging of substances – Agricultural workers may be exposed to harmful amounts of pesticides during the application in the field.

B) Environmental toxicology

Environmental toxicology deals with the potentially deleterious impact of chemicals, present as pollutants of the environment, to living organisms. Ecotoxicology has evolved as an extension of environmental toxicology. It is concerned with the toxic effects of chemical and physical agents on living organisms, especially in populations and communities with defined ecosystems.

C) Clinical toxicology

Clinical toxicology deals with diagnosis and treatment of the normal diseases or effects caused by toxic substances of exogenous origin i.e. xenobiotics.

D) Forensic toxicology

Forensic toxicology closely related to clinical toxicology. It deals with the medical and legal aspects of the harmful effects of chemicals on man, often in post mortem material, for instance, where there is a suspicion of murder, attempted murder or suicide by poisoning.

III. Based on the organ/system effect

1. Cardiovascular toxicology
2. Renal toxicology
3. Central nervous system toxicology
4. Gastrointestinal toxicology
5. Respiratory toxicology

5. **Toxicokinetics and Toxicodynamics**

Toxicokinetics deals with absorption, distribution, biotransformation (biotransformation) and excretion of chemicals.

Toxicodynamics deals with the biochemical and physiological effects of chemicals to the body and mechanisms of their actions.

A. Toxicokinetics

i) Absorption

Absorption is the process by which the chemical enters the body. It depends on the route of administration, dissociation (to become ionized), dissolution (ability of solid dosage form to become soluble), concentration, blood flow to the site, and the area of the absorptive site.

The common sites of absorption (routes of exposure) are

-Oral route – the GIT is the most important route of absorption, as most acute poisonings involve ingestions.

-Dermal route – lipid solubility of a substance is an important factor affecting the degree of absorption through the skin.

-Inhalational route – toxic fumes, particulate and noxious gases may be absorbed through the lungs.

-Bioavailability is the fraction of unchanged drug reaching the systemic circulation following of non-vascular administration. Therefore, a portion of the chemical fails to reach the systemic circulation in original form after oral administration

ii) Distribution

Distribution-is defined as the apparent volume into which a substance is distributed. Volume of distribution (Vd) is calculated from the dose taken and the resulting plasma concentration:

Vd = dose /plasma concentration

The importance of volume of distribution in toxicology is

- Predicting peak blood concentration of the chemical taken
- Calculating the amount of substance in the body to verify the quantity ingested
- Deciding whether to apply systemic toxin elimination techniques

Factors determining the rate of distribution of chemicals in the body are

-Protein binding – chemicals highly bound to protein have small volume of distribution

-Plasma concentration – when the volume of distribution of chemicals is small, most of the chemical remains in the plasma

-Physiological barriers – chemicals will not uniformly distributed to the body due to specialized barriers e .g blood brain barrier

-Affinity of chemicals to certain tissues – the concentration of a chemical in certain tissues after a single dose may persist even when its plasma concentration is reduced e .g Lead concentrate in bone tissue E .g A 60Kg epileptic victim attempted suicide by ingesting Phenytoin tablets. Vd listed is 0.6 L/Kg. Peak blood concentration measured by the laboratory is 50mg/ L. What is the dose of the drug that was taken by the victim?

Dose=plasma concentration x Vd

=50mg/L x (0.6L/Kg x 60Kg)

= 1800mg

iii) Biotransformation (metabolism)

Biotransformation is the biochemical transformation of a chemical. It is a process by which the body transforms a chemical and makes it more water soluble so the chemical can be eliminated more rapidly via the kidney into the urine. Biotransformation can produce metabolites that are pharmacologically active and toxic E.g. parathion parathoxon (toxic metabolite). Liver is the major site of biotransformation for many chemicals & other organs that are involved are lungs, kidneys, skin &so on. Interactions during biotransformation includes There are two phases of biotransformation

Phase I – the drug is converted into more polar compound e .g oxidation, reduction, &hydrolysis

Phase II (conjugation) – a drug or its metabolite is conjugated with an endogenous substance e .g glucuronide conjugate Enzyme inhibition- by this the biotransformation of drugs is delayed & is a cause of increased toxicity

Enzyme induction- by this the biotransformation of drugs is accelerated & is a cause of therapeutic failure

First – pass effect – is the biotransformation of some chemicals by the liver during the initial pass from the portal circulation after oral administration.

Half life (t ½) –is the time required to reduce the blood concentration of the chemical to half.

Principles of xenobiotic biotransformation

It is difficult to make categorical statements about xenobiotic biotransformation because there is an exception to every rule. Nevertheless, the following points, which might be considered principles or rules, apply in the majority of cases:

Point 1- Xenobiotic biotransformation or drug metabolism is the process of converting lipophilic (fat soluble) chemicals, which are readily absorbed from the gastrointestinal tract and other sites, into hydrophilic (water soluble) chemicals, which are readily excreted in urine or bile. There are exceptions even to this most basic rule. For example, acetylation and methylation are biotransformation reactions that can actually decrease the water solubility of certain xenobiotics.

Point 2- The biotransformation of xenobiotics is catalyzed by various enzyme systems that can be divided into four categories based on the reaction they catalyze:

1. Hydrolysis (e.g., carboxylesterase)
2. Reduction (e.g., carbonyl reductase)
3. Oxidation (e.g., cytochrome P450)
4. Conjugation (e.g., UDP-glucuronosyltransferase)

Point 3- In general, individual xenobiotic-biotransforming enzymes are located in a single organelle. However, in such cases, the enzyme name generally refers to two or more enzymes, each with its own distinct subcellular location. For example, the epoxide hydrolase located in microsomes is a different enzyme from the epoxide hydrolase located in cytosol (i.e., they are distinct gene products). From a practical perspective, it is noteworthy that during the homogenization of tissue and the preparation of subcellular fractions, a certain degree of cross-contamination of organelles occurs. For example, microsomes contain detectable levels of monoamine oxidase due to their contamination with the outer mitochondrial membrane.

Point 4- In general, xenobiotic biotransformation is accomplished by a limited number of enzymes with broad substrate specificities. In humans, for example, two cytochrome P450 enzymes— namely, CYP2D6 and CYP3A4—metabolize over half the orally effective drugs in current use.

The structure (i.e., amino acid sequence) of a given xenobiotic biotransforming enzyme may differ among individuals, which can give rise to differences in rates of drug metabolism.

Point 5- Hydrolysis, reduction, and oxidation expose or introduce a functional group (such as –OH, –NH2, –SH, or –COOH) that can be converted to a water-soluble conjugate. The first three reactions (hydrolysis, reduction, and oxidation) are often called Phase 1 reactions, and the conjugation reactions are often called Phase 2 reactions. The classification of xenobiotic biotransforming enzymes into Phase 1 and Phase 2 (and the extension of this system to classify xenobiotic transporters as Phase 3).

Point 6- Not all biotransformation reactions are catalyzed by the mammalian enzymes. Some biotransformation reactions are catalyzed by enzymes in the gut microflora (largely anaerobic bacteria in the colon), whereas the biotransformation of still other

xenobiotics is catalyzed by enzymes that participate in intermediary (endobiotic) metabolism.

Some drugs are intentionally designed to be biotransformed by endobiotic-metabolizing enzymes. For example, the anti-HIV drug zidovudine (AZT) is converted to a triphosphate nucleoside by enzymes (nucleoside kinase, nucleoside monophosphate kinase or NMK, and nucleoside diphosphate kinase or NDK).

Point 7- Several xenobiotic-biotransforming enzymes are inducible, meaning their expression can be increased (upregulated) usually in response to exposure to high concentrations of xenobiotics.

Point 8- Xenobiotic biotransformation can alter the biological properties of a xenobiotic. It can make the xenobiotic less toxic (detoxication), but in some cases it can make it more toxic (activation). The oxidation of ethanol (alcohol) to acetaldehyde is an example of xenobiotic activation, and the subsequent oxidation of acetaldehyde to acetic acid is an example of detoxication.

Point 9- In many cases, the toxicity of a xenobiotic is due to the parent compound (the compound that was absorbed), in which case xenobiotic biotransformation serves as a detoxication mechanism.

However, xenobiotic-biotransforming enzymes can convert certain xenobiotics to reactive (electrophilic) metabolites, and this activation process plays an important role in chemical toxicity and chemical mutagenicity/carcinogenicity.

Point 10- The balance between activation and detoxication by xenobiotic-biotransforming enzymes is often a key determinant of chemical toxicity, and is often the basis for organ or species differences in toxicity. For example, aflatoxin is converted by liver microsomal cytochrome P450 to a reactive epoxide that is thought to be

responsible for the hepatotoxic and hepatocarcinogenic effect of this mycotoxin. The fact that this reaction occurs in the liver explains why aflatoxin causes liver toxicity and liver tumors. On this basis, mice would be expected to be more sensitive than rats to the hepatotoxic effects of aflatoxin because mice catalyze the epoxidation of aflatoxin faster than rats.

Point 11- Although the small intestine and liver contain the highest concentrations, xenobiotic-biotransforming enzymes are nevertheless widely distributed throughout the body.

Point 12- Genetic variation in a xenobiotic-metabolizing enzyme produces four discernable phenotypes: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultrarapid metabolizers (UM).

IV) Excretion

Excretion is the final means of chemical elimination, either as metabolites or unchanged parent chemical. Excretion through the lungs is the major route for gaseous substances; and in the case of non-volatile water – soluble drugs, the kidneys are the most important routes of excretion. Additional routes include sweat, saliva, tears, nasal secretions, milk, bile and feces.

Clearance – elimination of chemicals from the body may be described by the term clearance (CL). It is a quantitative measure of the volume of blood cleared of drug per unit time, usually expressed in milliliter pe4r minute.

Clearance is calculated as follows

CL = 0.7 (VD)/ (t1/2) = ml/min

Where the VD is expressed in milliliter per kilogram & the half-life is expressed in minutes or hours.

Certain points regarding the toxicokinetics of toxic agents

- Drug absorption after a toxic ingestion may be delayed and prolonged;
- The half-life and total body clearance are often lengthened
- Liver-metabolizing enzymes may become saturated, slowing hepatic elimination;
- Chemicals with large volumes of distribution are often highly tissue-bound and measures to enhance their elimination are not effective;
- Poor perfusion of the liver and kidneys secondary to the toxic effects of the substance may slow clearance.

B. Toxicodynamics

Toxicodynamics is the mechanism of action of a toxic chemical to the body (what chemicals do to the body). The targets for the toxicodynamic actions of toxic chemicals are

- Enzymes
- Membrane receptors
- Intracellular receptors
- Ion channel

Toxic effects generally result from adverse cellular, biochemical, or macromolecular changes which attained by

- Damage to an enzyme system
- Disruption of protein synthesis
- DNA damage
- Modification of an essential biochemical function

Dose-response

The general dose-response principles are of crucial importance in determining the severity of the intoxication. We have two types of responses so called Quantal dose response (all- or – none response) & graded dose response (when dose increases, the response increases in graded fashion). Both responses show a typical dose response relation. The parameters that are derived from the dose response relationships are

Median lethal dose (LD50) – is the dose which is expected to kill 50% of the population in the particular group.

Median effective dose (ED50) –is the dose that produces a desired response in 50% of the test population when pharmacological effects are plotted against dosage.

Median toxic dose (TD50) – is the dose which is expected to bring toxic effect in 50% of the population in the particular group.

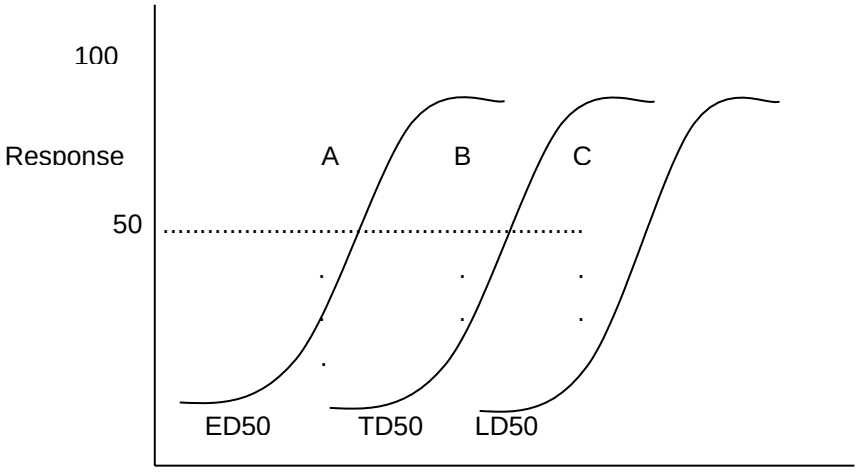

Fig. Comparison of dose –response curves for efficacy (A), toxicity (B), & lethality (C). The effective,toxic, &lethal dosage for 50% of the population in the groupcan be estimated as shown.

Common effects of chemicals to cause symptoms

Chemicals can cause symptoms through the following mechanisms

1.Interfere with the transport or tissue utilization of oxygen (carbon monoxide, cyanide), resulting in hypoxia or a decrease in an essential substrate such as glucose.

2.Depress or stimulate the CNS, producing coma (sedative- hypnotics) or convulsions (Sympathomimetics such as cocaine, amphetamines)

3.Affect the autonomic nervous system, producing cholinergic action (organophosphate insecticide)

4.Affect the lungs by aspiration (hydrocarbon)

5.Affect the heart and vasculature producing myocardial dysfunction, dysrrhythmias (antiarrhythmic agents) and hypertension or hypotension

6.Produce local damage (caustics and corrosives)

7.Delayed effects on the liver (acetaminophen) or kidneys (heavy metals.

6. **Potential sources of toxicities**

The potential causes of toxicities include

-**Therapeutic agents** –drug toxicity can be due to over doses, unusual adverse effects, frequent administrations of therapeutic doses & drug interactions

-**Industrial chemicals**- these chemicals may contribute to environmental pollution & they may be a direct hazard in the work place they are used.

-**House-hold chemicals** – The top household products ingested are cleaning agents, cosmetics & personal products & berries.

-**Environmental contaminants-** main sources of pollution to the environment are industrial processes, pesticides & smokes from factories & vehicles. Environmental pollutants may be released into the air, water, or dumped onto land.

-**Natural toxicants-** many plants & animals produce toxic substances for both defense & offensive purposes. Natural toxins may feature in poisoning via containing in food, by accidental ingestions of poisonous plants or animals & by stinging & biting

-**Food additives** – have usually low biological activity. Many different additives are added to food to alter the flavor or colour, prevent spoilage or in some other way change the nature of the food stuff. There are also many potentially toxic substances which are regarded as contaminants.

-**Traditional medicines (Botanicals)** – the medical use of botanicals in their natural &unprocessed form undoubtedly noticed long time ago. The use of botanicals has increased dramatically. Unfortunately, misconceptions regarding safety &efficacy of the agents are common. In fact, these products can be adulterated, misbranded or contaminated. Furthermore, the doses for active botanical substances may be higher. Adverse effects have been documented for a variety of botanical medications.

-**Drugs of abuse** - Excessive or improper use of drugs or other substances for non-medical purposes, usually for altering consciousness but also for body building is known as abuse of drug. There are a lot of drugs of abuse with high potential of dependence & tolerance (e.g alcohol, nicotine…)

7. Environmental considerations

Certain chemical and physical characteristics are known to be important for estimating the potential hazard involved for environmental toxicants. In addition to information regarding effects on different organisms, knowledge about the following properties is essential to predict the environmental impact:

The degradability of the substance; its mobility through air, water and soil; whether or not bioaccumulation occurs; and its transport and biomagnification through food chains.

If the intake of a long-lasting contaminant by an organism exceeds the latter's ability to metabolize or excrete the substance, the chemical accumulates within the tissues of the organism (e .g DDT). This is called bioaccumulation.

Although the concentration of a contaminant may be virtually undetectable in water, it may be magnified hundred or thousand time as the contaminant passes up the food chain. This is called biomagnification.

Chemicals that are poorly degraded (by abiotic or biotic pathways) exhibits environmental persistence and thus can accumulate. Lipophilic substances tend to accumulate in body fat, resulting in tissue residues. When the toxicant is incorporated in to the food chain, biomagnification occurs as one species feed upon others and concentrates the chemical. The pollutants that have the widest environmental impact are poorly degradable & relatively mobile in air, water and soil, exhibits bioaccumulation; and also exhibits biomagnification.

In ecotoxicology there are three interacting components; the toxicant, the environment and the organisms.

8. Poison prevention &control strategies

1.Keep all household poisons separate from food.

2.Keep all products in their original containers

3.Always read all labels carefully before using the product

4.Never give or take any medication in the dark

5.Dispose all products in a safe and proper manner

6.Encourage periodic home hunts and dispose of old medicine

7.Teach children never to take medication unless given by an adult they know and trust

8.Buy only those drugs supplied in childproof packaging

9.Once a child has been poisoned, be on the alert for repeat episodes

10.Teach children not eat plants or berries

11.Store all drugs or potentially toxic substances out of sight and out of reach of children: use cabinet lock

CLINICAL TOXICOLOGY LABORATORY

Introduction

Clinical toxicology involves the detection and treatment of poisonings caused by a wide variety of substances, including household and industrial products, animal poisons and venoms, environmental agents, pharmaceuticals, and illegal drugs. The toxicology laboratory must provide appropriate testing in three general areas: Identification of agents responsible for acute or chronic poisoning; Detection of drugs of abuse; and therapeutic drug monitoring. Increasingly sophisticated analytic methods are available to accomplish these tasks, but it is imperative that they be used judiciously. The numbers of compounds for which true emergency laboratory results are needed to guide therapy are still relatively few. For most potentially lethal intoxications the victim must be treated empirically before the laboratory results are known. A wide held misconception is that the laboratory can routinely detect any of the thousands of potential drugs or toxins that may be present in a sample. Because the financial and personnel resources required for such complete "screens" would be prohibitive, clinical laboratories must employ selective procedures suitable for the victim population in question. Therefore in most cases in clinical or hospital-based settings, tests are done for only a finite number of compounds, generally the more common drugs of abuse. Ideally, a diagnosis of poisoning would be made clinically, with the laboratory playing a confirmatory role. This short chapter is meant to discuss the basic structures which are said to be vital in clinical toxicology laboratory.

I. The role of clinical toxicology laboratory

Most poisoned victims can be treated successfully without any contribution from the laboratory other than routine clinical biochemistry and hematology. This is particularly true for those cases where there is no doubt about the poison involved and when the results of a quantitative analysis would not affect therapy. However, toxicological analyses can play a useful role

- If the diagnosis is in doubt,
- The administration of antidotes or protective agents is contemplated, or
- The use of active elimination therapy is being considered.
- Drug monitoring

II. Basic information necessary for toxicology laboratory

Close communication between clinical and laboratory personnel is essential. At a minimum, the ordering requisition for a toxicology screen should contain the following information.

A. Suspected agent(s)

The content of toxicology screens varies among laboratories. Although a standard screen may not include the suspected agent, if alerted beforehand the laboratory may be able to modify procedures as needed in order to search for the suspected agents.

B. Suspected dose

Analytic sensitivities vary among laboratories, and some facilities may not be able to detect therapeutic concentrations of certain drugs in their routine screens. Knowledge of the approximate dose ingested is important because in certain cases the use of analytic methods designed for therapeutic monitoring, not screening may be necessary.

C. Time of ingestion and sampling

Knowledge of both ingestion and sampling time is necessary to determine the degree of drug absorption; with serial determinations, knowledge of sampling times is critical, as a single quantitative level may be misleading and must be correlated with the time of ingestion. Serial levels, timed appropriately with respect to the pharmacokinetics of the agent, document that the concentration has peaked, which helps guide further therapy.

D. Clinical presentation

Knowledge of the clinical presentation helps the laboratory select the most appropriate screening procedures. The screening procedure for a victim with a depressed level of consciousness is entirely different from conscious victim.

E. Location of the victim

Location of the victim to the clinical laboratory determines the type of the test that is going to be done (e. g depending on its simplicity).

III. Steps in undertaking an analytical toxicological investigation

The analysis dealings with a case of poisoning are usually divided into pre-analytical, analytical and post-analytical phases

Pre-analytical phase

-Obtain details of current admission, including any circumstantial evidence of poisoning and results of biochemical and hematological investigations

-Obtain victim's medical history, if available, ensure access to the appropriate sample(s), and decide the priorities for the analysis.

Analytical phase

- Perform the agreed analyses.

Post-analytical phase

- Interpret the results and discuss them with the clinician looking after the victim.
- Perform additional analyses, if indicated, on the original samples or on further samples from the victim.

IV. Laboratory specimens

Before starting an analysis it is important to obtain as much information about the victim as possible (medical, social and occupational history, treatment given, and the results of laboratory or other investigations). It is also important to be aware of the time that elapsed between ingestion or exposure and the collection of specimens, since this may influence the interpretation of results.

No single specimen type is universally appropriate for identification of toxic agents. The selection of specimen type is based on both the toxicokinetics of the suspected agent and laboratory methodology. In general, quantitative tests are performed on serum or whole blood, and qualitative tests are performed on urine and gastric contents. When in doubt, obtain as many specimen types as possible and forward to the laboratory, where the most appropriate specimens can be selected. For the broadest possible screening (which, again, is rarely needed, especially in emergency toxicology), minimally, blood and urine should be sent.

A. Specimen collection

Urine

Urine is useful for screening tests as it is often available in large volumes and usually contains higher concentrations of drugs or other poisons than blood. The presence of metabolites may sometimes assist identification. A 50-ml specimen from an adult, collected in a sealed, sterile, plastic container, is sufficient for most purposes; no preservative should be added. Urine can be collected in acid washed, metal free container for quantification of heavy metals. The sample should be obtained as soon as possible, ideally before any drug therapy is initiated. Conversely, little poison may remain in specimens taken many hours or days later, even though the victim may be very ill, as in the case acute paracetamol poisoning.

Stomach contents

Stomach contents may include vomit, gastric aspirate and stomach washings - it is important to obtain the first sample of washings, since later samples may be very dilute. A volume of at least 20 ml is collected in plastic container to carry out a wide range of tests; no preservative should be added. It is the best sample on which to perform certain tests. If obtained soon after ingestion, large amounts of poison may be present while metabolites, which may complicate some tests, are usually absent. An immediate clue to certain compounds may be given by the smell; it may be possible to identify tablets or capsules simply by inspection.

Scene residues (non-biological)

It is important that all bottles or other containers and other suspect materials found with or near the victim (scene residues) are retained for analysis if necessary since they may be related to the poisoning episode.

A few milligrams of scene residues are usually sufficient for the tests described here. Dissolve solid material in a few milliliters of water or other appropriate solvent. Use as small amount as possible in each test, in order to conserve sufficient amount for possible further tests.

Blood

Blood (plasma or serum) is normally reserved for quantitative assays but for some poisons, such as carbon monoxide, whole blood has to be used for qualitative tests. Specimen should be collected in a sealed heparinized tube on admission. In addition, 2-ml sample should be collected in a fluoride/oxalate tube. The use of disinfectant swabs containing alcohols (ethanol, propan-2-ol) should be avoided. In general, there are no significant differences in the concentrations of poisons between plasma and serum.

B. Specimen transport and storage

Specimens sent for analysis must be clearly labeled with the victim's full name, the date and time of collection, and the nature of the specimen. This is especially important if large numbers of victims have been involved in a particular incident, or a number of specimens have been obtained from one victim. The date and time of receipt of all specimens by the laboratory should be recorded and a unique identifying number assigned to each specimen. Containers of volatile materials, such as organic solvents, should be packaged separately from biological specimens to avoid the possibility of cross-contamination. All biological specimens should be stored at 4°C prior to analysis. Ideally any specimen remaining after the analysis should be kept at 4°C for 3-4 weeks in case further analyses are required. In view of the medicolegal implications of some poison cases (for example, if it is not clear how the poison was administered or if the victim dies) then any specimen remaining should be kept (preferably at -20°C) until investigation of the incident has been concluded.

C. Specimen examination Urine

High concentrations of some drugs or metabolites can impart characteristic colors to urine. Treatment given for poisoning may color urine (E.g. Deferoxamine in iron poisoning color urine red or methylene blue given in treatment of nitrate poisoning may color urine blue). Strong-smelling poisons such as methylsalicylate can sometimes recognized in urine since they are excreted in part unchanged. Turbidity may be due to underlying pathology (blood, microorganisms, casts, epithelial cells), or carbonates, phosphates or urates (in amorphous or microcrystalline forms). Such findings should not be ignored, even though they may not be related to the poisoning.

Stomach contents and scene residues

Some characteristic smells can be associated with particular poisons (e. g alcohol). Very low or very high pH may indicate ingestion of acid or alkali, while a green/blue color suggests the presence of iron or copper salts. Microscopic examination using a polarizing microscope may reveal the presence of tablet or capsule debris. Undegraded tablets or capsules and any plant remains or specimens of plants thought to have been ingested should be examined separately.

V. Apparatus, reference compounds & reagents

A. Apparatus

Analytical toxicology services can be provided in clinical biochemistry laboratories that serve a local hospital or accident and emergency unit. In addition to basic laboratory equipment, some specialized apparatus, such as that for thin-layer chromatography, ultraviolet and visible spectrophotometry and microdiffusion, is needed. A continuous mains electricity supply is not essential. No reference has been made to the use of more complex techniques, such as gas-liquid and high-performance liquid chromatography, atomic absorption spectrophotometry or immunoassays, even if simple methods are not available for particular compounds. Although such techniques are more selective and sensitive than many simple methods, there are a number of factors, in addition to operator expertise, that have to be considered before they can be used in individual laboratories.

The standards of quality of laboratory reagents and glassware and of consumable items such as solvents and gases needs to be considerably higher than for the tests described in this manual if reliable results are to be obtained. Additional complications, which may not be apparent when instrument purchase is contemplated, include the need to ensure a regular supply of essential consumables (gas chromatographic septa, injection syringes, chromatography columns, solvent filters, chart or integrator paper, recorder ink or fibre-tip pens) and spare or additional parts (detector lamps, injection loops, column packing

materials). The instruments must be properly maintained. Some drug-testing techniques are now available in kit form. For example, there are standardized thin-layer chromatography (TLC) drug screening systems. Similarly, immunoassay kits are relatively simple to use, although problems can arise in practice, especially in the interpretation of results. Moreover, they are aimed primarily at the therapeutic drug monitoring and drug abuse testing markets and, as such, have limited direct application in clinical toxicology.

B. Reference compounds and reagents

A supply of relatively pure compounds for use as reference standards is essential if reliable results are to be obtained. However, expensive reference compounds of a very high degree of purity, such as those marketed for use as pharmaceutical quality control standards, are not normally needed. Some drugs, such as barbiturates, caffeine and salicylic acid, and many inorganic and organic chemicals and solvents are available as laboratory reagents with an adequate degree of purity through normal laboratory chemical suppliers. Such a reference collection is a valuable resource, and it should be stored under conditions that ensure safety, security and stability.

Although the apparatus required to perform the tests described in this manual is relatively simple, several unusual laboratory reagents are needed in order to be able to perform all the tests described. At last, it is beyond the scope of the lecture note to cover all the reagents.

VI. General laboratory tests in clinical toxicology

Many clinical laboratory tests can be helpful in the diagnosis of acute poisoning and in assessing prognosis. More specialized tests may be appropriate depending on the clinical condition of the victim, the circumstantial evidence of poisoning and the past medical history.

A. Biochemical tests

Blood glucose:

Determination of blood glucose is essential to know those toxic substances that affect blood glucose biotransformation. A toxicant that causes hypoglycemia includes insulin, iron, acetyl salicylic acid & so on.

Hyperglycemia is a less common complication of poisoning than hypoglycemia, but has been reported after over dosage with acetylsalicylic acid, salbutamol and theophylline.

Electrolytes, blood gases and pH

Toxic substances or their metabolites, which inhibit key steps in intermediary biotransformation, are likely to cause metabolic acidosis owing to the accumulation of organic acids, notably lactate.

Measurement of the serum or plasma anion gap can be helpful. The anion gap is usually calculated as the difference between the sum of sodium & potassium concentration and the sum of the chloride and bicarbonate concentrations ((Na++k+) + (Cl- + HCO3). It is normally about 10mmol/l.

If arterial blood gas measurement is performed, direct measurement of oxygen saturation with a CO-oximeter allows detection of methemoglobin, resulting from intoxication with various oxidizing drugs or Carbon monoxide-hemoglobin

Plasma enzymes

The plasma activities of liver enzymes, such as aspartate aminotransferase, alanine aminotransferase may increase rapidly after absorption of toxic doses of substances that can cause liver necrosis, notably paracetamol, carbon tetrachloride, and copper salts.

Cholinesterase activity

Plasma cholinesterase is a useful indicator of exposure to organophosphorus compounds or carbamates, and a normal plasma cholinesterase activity effectively excludes acute poisoning by these compounds.

The diagnosis can sometimes be assisted by detection of a poison or metabolite in a body fluid, but the simplest method available is relatively insensitive.

Measurement of serum osmolality

The normal osmolality of plasma (280-295mOsm/Kg) is largely accounted by sodium, urea &glucose. However, large increases in plasma osmolality may follow the absorption of osmotically active poisons (especially methanol, ethanol, or propan-2-ol) in relatively large amounts. Together with the standard chemistry panel, serum osmolality allows identification of an osmolal gap, which may indicate intoxication with ethanol or other alcohols.

B. Hematological tests

Hematocrit (Erythrocyte volume fraction)

Acute or acute-on-chronic over dosage with iron salts, acetylsalicylic acid, indomethacin, and other non-steroidal anti- inflammatory drugs may cause gastrointestinal bleeding leading to anemia.

Anaemia may also result from chronic exposure to toxins that interfere with haem synthesis, such as lead.

Leukocyte count

Increases in the leukocyte (white blood cell) count often occur in acute poisoning, for example, in response to an acute metabolic acidosis, resulting from ingestion of ethylene glycol or methanol, or secondary to hypostatic pneumonia following prolonged coma.

Blood clotting

The prothrombin time and other measures of blood clotting are likely to be abnormal in acute poisoning with rodenticides such as Coumarin anticoagulants.

Carboxyhemoglobin

Measurement of blood carboxyhemoglobin can be used to assess the severity of acute carbon monoxide poisoning. However, carboxyhemoglobin is dissociated rapidly once the victim is removed from the contaminated atmosphere, especially if oxygen is administered, and the sample should therefore be obtained as soon as possible after admission. Even then, blood carboxyhemoglobin concentrations tend to correlate poorly with clinical features of toxicity.

Common analytical toxicology laboratory techniques

I. Spot tests

Spot tests are rapid, easily performed, non-instrumental qualitative procedures. They are the most rudimentary toxicology tests, & generally performed on urine specimens. In the test procedure, the sample (that is suspected for having a particular toxic chemical) will react with a chemical or chemicals set as a solution, or coated on a strip & the result of the reaction expressed by a color formation detected visually or colorometrically.

Spot tests are available for a number of compounds, including salicylate, acetaminophen, carbonmonoxide, halogenated hydrocarbons, and heavy metals. The tests are rapid and convenient; however sensitivity and specificity are generally poor and accurate quantification is virtually impossible. Because of improvements in other technologies, spot tests are now largely replaced by rapid immuno- assays that may perform at the point- of-care or in the central laboratories.

II. Ultraviolet & visible spectrophotometry

Many toxins have characteristic absorption spectra, but they must be extracted from body fluids in order to measure these spectra. A number of the quantitative methods employ ultraviolet (UV) (200-400 nm) or visible (400-800 nm) spectrophotometry. The major problem encountered with this technique is interference, and some form of sample

purification, such as solvent extraction or microdiffusion, is usually employed. For some drugs (e.g., barbiturates, benzodiazepines and theophylline) the method offers reasonable sensitivity and specificity, but it is much less powerful and versatile than chromatographic method.

III. Immunoassays

Immunoassays are diagnostic techniques used for the detection of antigen and antibody. Depending on the immunoassay techniques that are employed for the specific test, either antigen or antibody may be detected from the samples based on their reaction with their specific antibody or antigen respectively.

Many types of immunoassay configuration can be devised. Those not involving radioactivity or separation steps (homogeneous immunoassays) can be automated on routine clinical chemistry instruments, making them convenient for laboratories of all sizes. Immunoassay techniques used to screening specimens for chemicals include: Enzyme-Multiplied immunoassay (EMIA), Florescence polarization Immunoassay (FPIA), Cloned enzyme donor Immunoassay (CEDIA), and Radio Immunoassay (RIA). Immunoassays can be made highly sensitive and quite specific, but their specificity is never absolute. Molecules with a similar structure generally cross-react to some degree, and occasionally substances interfere with the assay in some other fashion.

Immunoassays also have the drawback that each analyte must be individually assayed using an available antibody reagent. Nevertheless, some of the more modern, discrete analyzers can readily perform multiple homogenous immunoassays with minimal operator intervention, so a panel of commonly abused drugs (e.g., barbiturates, cocaine, opiates, cannabinoids, amphetamines, benzodiazepines) can be readily tested.

Immunoassay techniques have also been modified for on-site testing in the emergency department and other out victim settings. These tests are known as drug dipsticks; and they utilize paper strips impregnated with drug-specific antibody. The specimen is applied to the paper, and reagents produce a color development.

IV. Chromatography

Chromatography is a powerful technique for separating substances based on slight differences in chemical properties. In this method, components to be separated are distributed between two phases; as stationary and mobile phases.

Chromatographic procedure involve a sample to be introduced in a flowing stream of gas or liquid (mobile phase) that pass through a bed, layer, or column containing a stationary phase (made from solid, or gel or a liquid). As the mobile phase carries the sample pass the stationary phase, the solutes with lesser affinity remain in the mobile phase & travel faster & separate from those that have great affinity for it. Different chemicals have different characteristic mobility in a particular chromatographic system, allowing fairly confident identification.

In contrast to immunoassays, small chemical changes (e.g., addition or removal of a methyl group), commonly cause substantial changes in chromatographic mobility. Thus the parent drug can usually be distinguished from its metabolites.

Types of chromatographic techniques

a. Thin-layer chromatography (TLC)

TLC has been widely used for urine toxicology. It does not require special equipment, is suitable for analysis of large batches of samples, is available in commercial kit form, and allows use of various color reagents in addition to chromatographic mobilities to aid in chemical identification. TLC, however, is too slow and cumbersome to be readily applied to emergency toxicology, and it is generally not quantitative. Its sensitivity is relatively poor.

b. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are powerful techniques requiring dedicated equipment and skilled operators, but they can be adapted to a wide range of screening or quantitative assays.

Gas chromatography

GC is the technique of choice for volatile agents (ethanol, methanol, isopropanol, ethylene glycol). Use of open tubes (capillaries) allows rapid, high-resolution separations. Many detection methods can be applied, some with high sensitivity; and definitive drug identification is possible by coupling the GC with mass spectrometry.

c. HPLC

HPLC, which was developed more recently than GC, is a more natural technique for the analysis of nonvolatile compounds. Modern columns perform highly efficient separations, although resolution is not as good as that of GC. Detection is usually by ultraviolet spectrophotometry, which in its most sophisticated form permits spectral scanning of each eluting peak to aid in identification.

V. Mass spectrometry and other specialized techniques.

Mass spectrometry is an analytical instrument that first ionizes a target molecule and then separates and measures the mass of a molecule or its fragment. The analysis is qualitative, quantitative & extremely useful for determining the elemental composition & structure of both inorganic & organic compounds.

When mass spectroscopy (MS) is coupled to gas chromatography (GC-MS), nearly full proof chemical identification is possible because substances are identified from both their retention time measured by GC and their characteristic fragmentation pattern on MS. Using computer- based libraries of fragmentation patterns, GC-MS can be used to screen a wide variety of drugs simultaneously. Despite the availability of affordable bench-top instruments, however, GC- MS remains too sophisticated for routine application in clinical toxicology, although it has tremendous importance as the essential confirmatory technique in forensic toxicology.

VI. Atomic absorption, plasma emission, neutron activation, and x-ray fluorescence

Toxic metals, for which most of the previously discussed methods do not apply, can be analyzed by sophisticated spectroscopic techniques, including atomic absorption, plasma emission, neutron activation, and x-ray fluorescence.