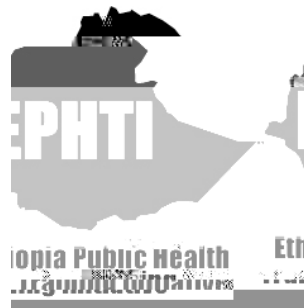
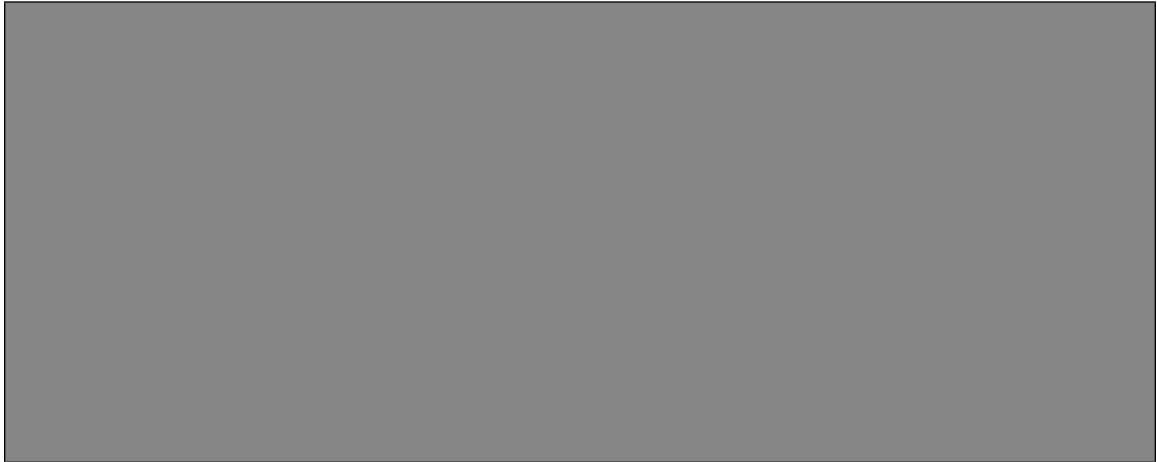






A

The scope of toxicology widened tremendously during the last few years. An important development in this discipline is mandatory because of the expansion of different industrial, medical, environmental, animal and plant noxious substances. So toxicology has got special attention to the deleterious effects of chemicals and physical agents on all living systems.

students to improve the quality of the diagnosis in poisoned patients.

The Chapters open with a guiding list of objectives & end up with questions to challenge the readers about the subject matter. Most of the sections have an introduction part designed to provide the background information of the materials to be covered.

Primary references to particular methods have not been given, in order to simplify presentation & also because many tests have been modified over the years, so that reference back to the original paper could cause confusion. For further information & supplementations, readers are supposed to

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ABB A

AAS- Atomic absorption spectroscopy

ABGs- Arterial blood gas analysis

ALA- D- Delta amino levulinate dehydratase

BUN- Blood urea & nitrogen

CBC- Complete blood count

CNS- Central nervous system

CO- Carbon monoxide

DDT – Dichloro-Diphenyl-Trichloroethane

EPP- Erythrocyte proto porphyrine

GC- Gas chromatography

GC-MS- Gas chromatography-Mass spectrometry

GIT- Gastrointestinal tract

HCl- Hydrochloric acid

HPLC- High performance liquid chromatography

NaOH- Sodium hydroxide

nm- nano meter

NMR- Nuclear magnetic resonance

PH- Power of hydrogen

PT- Prothrombin time

PTT- Partial prothrombin time

$t_{1/2}$ - Half-life

TLC- Thin layer chromatography

UV- Ultraviolet

Vd- Volume of distribution

CHAPTER ONE



has grown. So toxicology has a very important role to play in modern



Toxicology

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

The following toxicological data are derived from *American association of poison control center*. So it is mostly a description of the epidemiology of unintentional poisoning. It is very difficult to find the primary data of poisoning in our country because most of the screening & confirmatory tests are not done routinely in our set up. Additionally, we don't have well organized poison control center.

Today, 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



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)

Potentiation effect - the capacity of a chemical to increase the effect of another chemical without having the effect e .g Disulfiram at non-toxic doses potentiate the toxicity of alcohol & used in the treatment of alcohol abuse.

Antagonism





Toxicology

- 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 etc.

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

Toxicology

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 (V_d) is calculated from the dose taken and the resulting plasma concentration:

$$V_d = \text{dose} / \text{plasma concentration}$$

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



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 ($t_{1/2}$) – is the time required to reduce the blood concentration of the chemical to half.

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 per minute.

Clearance is calculated as follows

$$CL = 0.7 (V_D) / (t_{1/2}) = \text{ml/min}$$

Where the V

Toxicology

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

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 (see below). The

parameters that are derived from the dose response relationships are

Median lethal dose (LD50)



Dosage

Fig. Comparison of dose –response curves for efficacy (A), toxicity (B), & lethality (C). The effective, toxic, & lethal dosage for 50% of the



Toxicology

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

Toxicology

accumulate. Lipophilic substances tend to accumulate in body fat, resulting in tissue residues. When the toxicant is incorporated into the food chain, biomagnification occurs as one species feeds upon others and concentrates the chemical. The pollutants that have the widest environmental impact are poorly degradable & relatively mobile in air, water and soil, exhibit bioaccumulation; and also exhibit biomagnification.

In ecotoxicology there are three interacting components; the **toxicant**, the **environment** and the **organisms** (community, population or ecosystem).

8. Poison prevention & control strategies

- a) Keep all household poisons separate from food.
- b) Keep all products in their original containers
- c) Always read all labels carefully before using the product
- d) Never give or take any medication in the dark
- e) Dispose all products in a safe and proper manner
- f) Encourage periodic home checks and dispose of old medicine
- g) Teach children never to take medication unless given by an adult they know and trust
- h) Buy only those drugs supplied in childproof packaging
- i) Once a child has been poisoned, be on the alert for repeat episodes
- j) Teach children not to eat plants or berries
- k) Store all drugs or potentially toxic substances out of sight and out of reach of children: use cabinet locks

Exercise

1. Define toxicology & the terms used in toxicology.
2. Discuss the epidemiologic aspects of toxicology.
3. Discuss the basic classifications of toxicology.
4. Explain what toxicokinetics and toxicodynamics deals with.
5. Write some of the important environmental considerations in toxicology.
6. List some of the potential sources of toxicity.
7. Mention poisoning prevention & control strategies.

CHAPTER TWO





if a number of poisons with different actions have been absorbed. Moreover, many drugs have similar effects on the body, while some clinical features may be the result of secondary effects such as anoxia. Thus, if a victim is admitted with depressed respiration and pin-point pupils, this strongly suggests poisoning with an opioid.

Diagnoses other than poisoning must also be considered. For example, coma can be caused by a cerebrovascular accident, uncontrolled diabetes infections as well as poisoning. The availability of the results of urgent biochemical and hematological tests is obviously important in these circumstances.

Principles of management of poisoning

The initial management of a patient with altered mental status follows the follow the same approach regardless of the poison involved. The airway should be checked, breathing & circulation should be assessed. Then symptomatic & supportive measures are taken. After this, one can begin in a more detailed evaluation to make a specific diagnosis. Therefore, in principle, during poisoning, one should treat the victim first followed by treating the poison itself.

General measures

1. Supportive measures

The first priority is to establish & maintain vital functions. Subsequently, most victims can be treated successfully using supportive care alone.

- Maintain air way, adequate ventilation & oxygenation, provide tracheal intubation if required
- If comatose, administer glucose, thiamine, & oxygen
- For seizures, administer anticonvulsants

2. Principles of toxin eliminations

- If the poison has been inhaled, the victim should first be removed from the contaminated environment.
- If skin contamination has occurred, contaminated clothing should be removed and the skin washed with an appropriate fluid, usually water.
- In adult victims, gastric aspiration and lavage (stomach washout) are often performed, if the poison has been ingested, to minimize the risk of continued absorption.
- Similarly, in children emesis can be induced by the oral administration of syrup of ipecacuanha (ipecac).

Toxicology

- The absorption of any residue remaining after gastric lavage can be minimized by leaving a high dose of activated charcoal in the stomach.
- The role of lavage and induced emesis in preventing absorption is currently being examined, as is the effectiveness of a single dose of activated charcoal. However, repeated oral administration of activated charcoal appears to be effective in enhancing elimination of certain poisons.
- Specific therapeutic procedures, such as antidotal and active elimination therapy are sometimes indicated. The results of either a qualitative or a quantitative toxicological analysis may be required before some treatments are commenced because they are not without risk to the victim. In general, specific therapy is only started when the nature and/or the amount of the poison(s) involved are known. Antidotes or protective agents are only available for a limited number of poisons. In summary there are four main methods of enhancing elimination of the poison from the systemic circulation:
 1. Repeated oral activated charcoal;
 2. Forced diuresis with alteration of urine pH;
 3. Peritoneal dialysis and haemodialysis; and
 4. Haemoperfusion.

Some antidotes & protective agents used to treat acute poisoning

Antidote

Acetylcysteine

Indication

Paracetamol

Toxicology

Atropine

Deferoxamine

Methylene blue

Physiostigmine

Naloxone

Pyridoxine

Organophosphate

Iron

Nitrates

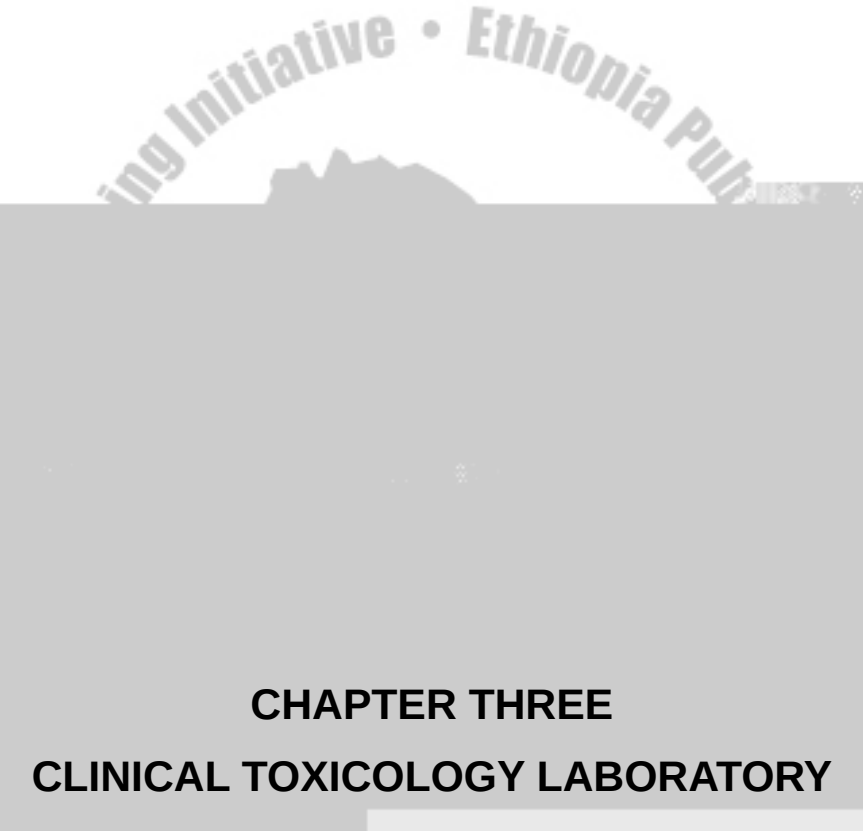
Atropine

Opioids

Isoniazid

Exercise

1. What are the common ways used for diagnosing poisoned victim?
2. Write the basic principles of management of poisoning



CHAPTER THREE

CLINICAL TOXICOLOGY LABORATORY

Learning Objectives

At the end of this chapter the student will be able to:

Toxicology

1. Mention the basic necessary information for clinical toxicology laboratory
2. Explain the role of clinical toxicology laboratory
3. Describe steps in undertaking analytic toxicological investigations
4. Discuss about collection, transportation, storage, characteristics, physical examination & analytical tests of laboratory specimens.
5. Describe about apparatus, reference compounds & reagents used in clinical toxicology laboratory
6. Discuss the routine laboratory tests carried out in clinical laboratory tests.

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



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

A. Suspected agent(s)

The content of toxicology screens varies among laboratories.



Toxicology

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



Toxicology

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

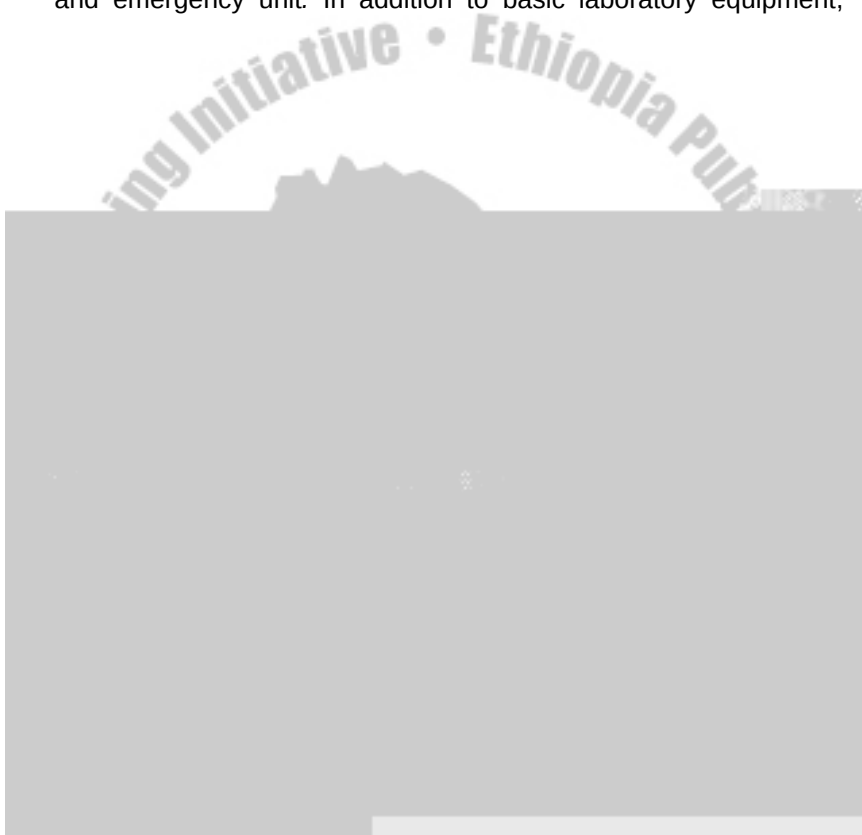
Blood (plasma or serum) is normally reserved for quantitative assays but for some poisons, such as carbon monoxide, whole blood has to be used for qualitative tests. Specimen should be



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 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 m-0.2 D2 (a)-0.2 (a) -0.2 (t) 0.2 (rn) -0.2 (i) -0.2 (n) 4.2 (,



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

If arterial blood gas measurement is performed, **direct measurement of oxygen saturation**



Toxicology

Osmolal gap can be calculated:

$$\text{Osmolal gap (Osmolarity)} = 2(\text{Na}^+) + \text{Glucose} \div 18 + \text{BUN} \div 2.8$$

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

Toxicology

obtained as soon as possible after admission. Even then, blood carboxyhemoglobin concentrations tend to correlate poorly with clinical features of toxicity.

Exercise

1. What is the basic information necessary for clinical toxicology laboratory?
2. What are the roles of clinical toxicology laboratory?
3. Mention the steps that are necessary to undertake analytic toxicological investigations.
4. Describe specimen collection, transportation, storage, characteristics & physical examination used in clinical toxicology laboratory.
5. Describe apparatus, reference compounds & reagents used in clinical toxicology laboratory.
6. Describe the routine laboratory tests used in clinical toxicology laboratory.

CHAPTER FOUR

PRACTICAL ASPECTS OF ANALYTICAL TOXICOLOGY

Learning Objectives

At the end of this chapter the student will be able to:

1. Define the methods used in practical aspects of analytical toxicology
2. Understand the common toxicology laboratory techniques

Introduction

Methods for particular toxicologic tests or panels are a well established part of routine laboratory tests, and information about them is available on request. In order to interpret toxicology results

A. Selection of test methods

Selection of test methods can be generally classified as either screening or confirmatory.

I. Screening methods

Screening is the testing or examining of a poisoned person for a chemical agent causing toxicity. Screening methods are generally qualitative, relatively simple and inexpensive, and designed to maximize sensitivity (possibly with some sacrifice of specificity). No standard toxicology screening tests exists. Currently the most widely used screening tests are based on immunoassay methods. Screening methods, give the emphasis on maximizing sensitivity, may produce significant numbers of false-positive results.

A “**negative**” **screen** can rule out only the finite number of compounds tested for at concentrations above the threshold of detection for the particular method used. Because of the inherent limitations of screening tests, definitive results must be based on a second method, a confirmatory procedure. It is important to note that inclusion of chemicals in a screening panel is generally governed by methodological as well as clinical considerations.

Reporting & interpretation of toxicology screening results

Toxicology screen results are usually reported with a list of the chemicals tested for and a comment regarding detection or presence of the chemicals (See annex III).

1. **Positive screens:** The notation “toxin detected” is entered next to the particular chemicals found.
2. **Negative screens:** The notation “toxin tested for not detected” or similar comment is made. Negative toxicology screen results

in the face of strong clinical suspicions to the contrary may occur due to a number of reasons.

- a. Toxins clinically suspected and in fact present in a victim are not **tested for**. Thus a seemingly negative toxicology screen result is misleading. If laboratory personnel are notified of the suspected agents, they can generally either modify the existing screen or suggest alternative strategies.

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

Toxicology

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

V. Mass spectrometry and other specialized techniques.

Mass spectrometry is an analytical instrument that first ionizes a



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CHAPTER FIVE

TOXICANTS OF PUBLIC HEALTH HAZARD

Learning objectives

At the end of this chapter the student will be able to: -

1. Understand industrial toxicants like lead, insecticides, rodenticides, cyanide& hydrocarbons with their



I. Industrial toxicants

Industrial chemicals causing diseases have existed ever since man began manufacturing on a large scale & during the industrial revolution occupational diseases became common. Many of the chemicals used in industry are chemically reactive molecules & are likely to interact with biological systems & cause damage in some cases at the site of exposure. Exposure is most commonly via skin & lungs. There are now many thousands of chemical substances used in industry ranging from metals & inorganic compounds which risk people who work with it.

A. Heavy metal poisoning

Some metals such as iron are essential for life, while others such as lead are present in all organisms but serve no useful biologic purpose. Some of the oldest diseases of humans can be traced to heavy metal poisoning associated with metal mining, refining and use. Heavy metals are found every where: including in food, air, water...

Lead Poisoning

Lead poisoning is one of the oldest occupational and environmental diseases in the world. Despite its recognized hazards, lead continues to have widespread commercial application (like ingested lead paints, pica, and lead pipes etc...). Environmental lead exposure, ubiquitous by virtue of the anthropogenic distribution of lead to air, water and food, has declined considerably due to diminished use of lead in gasoline and other applications. Lead serves no useful purpose in the human body. Lead is slowly but consistently absorbed via the

Toxicology

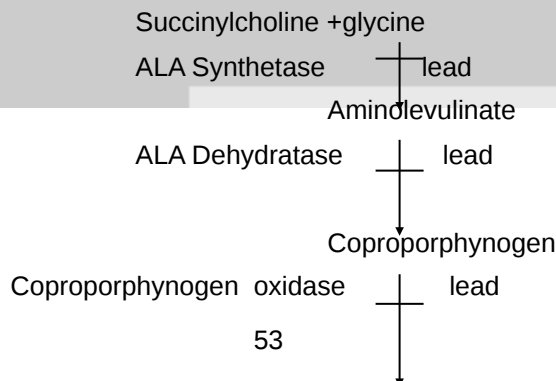
respiratory and gastrointestinal tracts. Inorganic lead is poorly absorbed through the skin. Absorption via the GIT varies with the nature of the lead compound, but in general, adults absorb about 10% of the ingested amount while young children absorb closer to 50%. The daily lead consumption is about 300µg. It is unsafe if consumed at a concentration greater than 0.5 mg/day for 3 months or more. Once absorbed from the respiratory or GIT, lead is bound to erythrocytes and widely distributed initially to soft tissues, then to the subperiosteal surface of bone and bone matrix. It has a half-life of 2-3 weeks in blood and 15 years in bone. More than 90% of the lead that is eliminated appears in the urine.

Lead exerts multi systemic toxic effects through at least three mechanisms by;

Inhibiting enzyme activity (e. g Interference with enzymes responsible for hemesynthesis) (See the fig below).

Interfering with the action of essential cations, particularly calcium, iron, and zinc.

Altering the structure of cell membranes and receptors (e. g attachment of lead to RBC membranes ! increased fragility and decreased survival time due to interference of sodium-potassium pump).



Protoporphyrin
Ferrochelase + iron — lead

Heme

Hemoglobin

Fig. Lead interference with the biosynthesis of heme

The sign and symptoms of lead poisoning may include anorexia, apathy, behavioral changes, persistent vomiting, convulsions (acute poisoning) & ataxia, wrist & ankle drop, chronic nephritis (chronic poisoning)

commonly utilized method. Levels below the toxic range do not rule out toxicity because 90% of lead is stored in bone. Unexpectedly high lead levels may be due to contamination of the blood specimen with lead prior to laboratory analysis. Sample must be taken with lead free needle and containers.

c) Erythrocyte Protoporphyrin (EPP)

EPP often referred free erythrocyte Protoporphyrin (FEP). Protoporphyrin accumulates as a result of the lead inhibition of the enzyme ferrochelases, which binds to porphyrin, forming hemoglobin. EPP is regarded as the foremost test for chronic lead poisoning. EPP performed in conjunction with blood lead levels to obtain more accurate picture. EPP is the most widely utilized screening test. A finger stick specimen can be used with a fluorometer to perform the test.

D) Delta-aminolevulinate dehydratase activity (ALA-D)

Lead decreases the activity of ALA-D, which is present in the erythrocytes. It is more sensitive than Protoporphyrin levels.

E) Urinary ALA and coproporphyrin III

Urinary levels of ALA are increased owing to lead inhibiting the enzyme ALA-D. Lead inhibition of the enzyme coproporphyrinogen oxidase has been proposed as a cause for increased coproporphyrin.

F) Calcium disodium versenate ($\text{CaNa}_2\text{-EDTA}$) provocation test

Specific laboratory tests

Qualitative test

Specimen

Stomach contents and scene residues

Reagents (see annex I)

1. Sodium tartrate buffer, pH 2.8.
2. Aqueous sodium rhodizonate solution (10 g/l).

Procedure

1. Add 0.1 ml of sodium tartrate buffer to 0.1 ml of test solution and vortex-mix for 5 seconds.
2. Spot 50 μ l of acidified solution on to phase-separating filter-paper and add 50 μ l of sodium rhodizonate solution.

Results

Lead salts give a purple colour in this test. However, the test is not specific: barium salts give a brown colour and a number of other metals also give coloured complexes.

Sensitivity

Lead, 2 mg/l

Quantitative tests

Principle

Whole blood that represents calibrators, controls, or victim specimens is mixed with ammonium phosphate and Triton X-100 to prepare it for graphite furnace atomic absorption analysis. The final step of analysis causes vaporization of lead, which absorbs energy at the 283.2nm light emitted from a hollow cathode lamp. Absorbance of energy at this wavelength is specific for lead and proportional to its concentration. Exposure of the specimen to high



5.



ordinary fuels are common examples. These agents were the most frequently involved substances in human exposures, accounting for almost 5% of all poisoning. The overall mortality rate for accidental ingestion of these agents is difficult to estimate but may approach 0.5%. Modes of toxicity vary with age. Most cases involve accidental imbibing by young children. Abuse by inhalation is generally seen in male adolescents & young adults. The most common substances reported in toxic ingestions are gasoline, kerosene, mineral seal oil preparations, & lighter fluid. Most victims who are exposed to hydrocarbon develop pulmonary symptoms due to aspiration pneumonitis. It is clear that non-pulmonary manifestations like CNS toxicity, GI signs, and cutaneous signs are distinctly uncommon.

LABORATORY STUDIES

- Routine laboratory tests are of little value for purposes of screening victims for admission.
-

1. sodium hydroxide solution(20%,w/v, aqueous)
2. Pyridine
3. Positive control (dissolve 500mg chloral hydrate in 100ml of ethanol.)

Procedure

1. Take 1ml of the urine sample in a test tube
2. Add 1ml of 20% NaOH & 1ml of pyridine
3. Heat in a boiling water bath for 1 minute
4. A pink red color in the pyridine layer indicates the presence of hydrocarbon.

C. PESTICIDES

Pesticides are any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticide can be divided into several groups, such as insecticides, rodenticides, fungicides & herbicides. This part will give attention to the most frequent pesticide poisoning.

1) Insecticides

Organophosphates & carbamates are the most frequently used insecticides world wide. These compounds cause 80% of the reported toxic exposure to insecticides.

Organophosphorus insecticides

These agents are utilized to combat a large variety of pests. Some of these agents are used in human and veterinary

compounds are absorbed by the skin as well as by the respiratory and GITs. Biotransformation is rapid. In mammals as well as insects, the major effect of these agents is inhibition of acetyl cholinesterase. The signs and symptoms that characterize acute intoxication are due to inhibition of this enzyme resulting in accumulation of acetylcholine (diarrhea, urination, miosis, bradycardia, lacrimations & salivation)

Laboratory analysis

1. Serum electrolytes (e.g. Hypokalemia, hyperglycemia), Blood urea nitrogen, creatinine, CBC (e.g. leukocytosis secondary to increased catecholamine release from the adrenal medulla), urinalysis (e.g. Proteinuria and glycosuria), amylase level (elevated due to pancreatic injury), plasma and RBC cholinesterase levels
2. Toxic – specific findings
 - Plasma and RBC cholinesterase levels are depressed
 - Depression of RBC cholinesterase level is more specific for

2. Cyclohexane:acetone:chloroform (70:25:5).
3. Acetone:tetraethylenepentamine (9:1).
4. 4-(*p*-Nitrobenzyl) pyridine (20 g/l) in acetone:tetraethylenepentamine (9:1).
5. Silica gel thin-layer chromatography plate (5 × 20 cm, 20 μm average particle size ;).

Procedure

1. Carefully adjust the pH of 10 ml of sample to about 7 by adding solid sodium bicarbonate.
2. Extract 10 ml of sample with 5 ml of methyl tertiary-butyl ether for 5 minutes using a rotary mixer.

30.2 (ra) -0a6diu1 25s (5mltftd -37.92 (r) 3522 (() -35 (f) 0.2 (o) 332 (O) -1650.7

Toxicology

Confirmatory test for organophosphorus pesticide is cholinesterase activity test.

Specimen

Plasma or serum

Cholinesterase activity monograph

Qualitative test

Specimen

Plasma or serum

Reagents (see annex I-number 7)

1. Dithiobisnitrobenzoate reagent. .
2. Aqueous acetylthiocholine iodide solution.
3. Aqueous pralidoxime chloride solution.
4. Plasma or serum from an unexposed individual (control plasma).

Procedure

1. Add 2.0 ml of dithiobisnitrobenzoate reagent and 1.0 ml of acetylthiocholine iodide solution to each of three 10-ml test-tubes.
2. Add 20 μ l of control plasma to one tube and 20 μ l of test plasma to a second.
3. Add 20 μ l of pralidoxime solution and 20 μ l of test plasma to the third tube.
4. Vortex-mix the contents of all three tubes and allow to stand at room temperature for 2 minutes.

Results

The presence of an acetylcholinesterase inhibitor is indicated if the yellow colour in the control tube is deeper than in the test tube. If the colour in the tube containing pralidoxime is similar to that in the control tube, this provides further confirmation that an inhibitor of acetylcholinesterase is present in the sample. Inhibitors of acetylcholinesterase, such as many carbamate pesticides, also give a positive result in this test

Treatment

GI decontamination

Dermal decontamination

Symptomatic treatment

Toxin-specific like atropine, pralidoxime

Carbamate pesticides

Produce a milder form of toxicity, similar to that produced by organophosphate compounds. These compounds inactivate acetylcholinesterase leading to excessive accumulation of acetylcholine. The important differences distinguishing carbamates from organophosphate toxicity are

- Carbamate toxicity is typically short-lived in which spontaneous regeneration of enzymatic activity usually occur within 24hours
- Carbamates produce little or no CNS toxicity because of their inability to penetrate the blood-brain barrier

RBC & plasma cholinesterase measurement not useful due to minimal & transient effects on these levels

Qualitative test

Specimen

Stomach contents, scene residues.

Reagents

1. Aqueous hydrochloric acid (2 mol/l)



Carbamates give a black spot. Non-pesticide carbamates can interfere in this test.

Sensitivity

Carbamate, 100 mg/l

Treatment

GI decontamination

Atropine, & pralidoxime can be used as an antidote

2. Rodenticides

Rodenticides are used to control rodent population. Anticoagulant preparations, currently the most widely used rodenticides, are safer, although consequential human poisonings do occur. Most pediatric ingestions occur accidentally, whereas ingestions in adults tend to be deliberate. Coumarin derivatives (E.g. warfarin) are one of the members of this class. Mechanism of action is by inducing coagulopathic state by inhibiting activation of the vitamin K – dependent clotting factors II, VII, IX and X. Victims are usually asymptomatic unless presentation is delayed over a period of several days, as the anticoagulant effects take place victims may experience spontaneous bleeding. The main features of warfarin poisoning in less severe cases are excessive bruising, nose & gum bleeding, & blood in the urine faeces. Bleeding from several organs within the body, leading to shock & possibly death, occurs in the more severe cases. The onset of the signs of

Laboratory analysis

- General tests
 - Check a baseline prothrombin time (PT), partial prothrombin time (PTT) and CBC
- Toxin-specific tests
 - Recommended monitoring factor VII-X complex levels are sensitive indicators of toxicity
 - Warfarin may be detected by gas chromatography

Specific laboratory tests

Quantitative test

Specimen

Urine, plasma

Procedure

HPLC method is used for the fluorometric determination of warfarin & its metabolites. The detection scheme utilizes post column acid-base fluorescence enhancement techniques that provide high chromatographic specificity & sensitivity. Detection limit are in the low nanogram range. Other procedures, like UV spectrometry & revised-phase liquid chromatography, with a detection limit in blood serum of 20 g/L.

D) CYANIDE TOXICITY

Cyanide is a cellular poison that can readily bind to many enzymes, having metallic component especially cytochrome oxidase a terminal enzyme involved in aerobic

biotransformation. Inhibition results in anoxia. Cyanide has



ii) Toxin – specific tests

1. A spot test is a quick bedside test that can qualitatively detect the presence of cyanide using gastric aspirate.
2. The specific cyanide level is the gold standard test and should be done even though the results may not be readily available. These levels are usually performed on whole blood but some laboratories use serum or plasma

Specific laboratory tests

Qualitative test

Specimen

Stomach contents, scene residues.

N.B:- specimens containing cyanides often evolve hydrogen cyanide if acidified.

Reagents

1. Aqueous sodium hydroxide solution (100 g/l).
2. Aqueous ferrous sulfate solution (100 g/l, freshly prepared in freshly boiled and cooled water).
3. Aqueous hydrochloric acid (100 ml/l).

Procedure

1. Dilute 1 ml of sample with 2 ml of sodium hydroxide solution.
2. Add 2 ml of ferrous sulfate solution.
3. Add sufficient hydrochloric acid to dissolve the ferrous hydroxide precipitate.

Result

Toxicology

A blue colour indicates the presence of cyanide.

Sensitivity

Cyanide, 10 mg/l

Quantitative assays

Specimen

Heparinized whole blood (0.1-1.0 ml),

N.B .The samples can be stored at 4°C for 1-2 days if the analysis is delayed for any reason. (Cyanide in blood is less stable if stored at room temperature or at -20°C.)

p-Nitrobenzaldehyde/ o-dinitrobenzene method

Reagents

1. Aqueous sodium hydroxide (0.5 mol/l).
2. Aqueous sulfuric acid (3.6 mol/l).
3. *p*-Nitrobenzaldehyde (0.05 mol/l) in 2-methoxyethanol.
4. *o*-Dinitrobenzene (0.05 mol/l) in 2-methoxyethanol.

Standard

Aqueous potassium cyanide (10 mg/l, i.e., cyanide ion concentration, 4 mg/l)

Method: microdiffusion method

Toxicology

1. Take three microdiffusion cells and add to each of the centre wells:
 - (a) 0.5 ml of *p*-nitrobenzaldehyde solution;
 - (b) 0.5 ml of *o*-dinitrobenzene solution;
 - (c) 0.1 ml of sodium hydroxide solution
2. To the outer wells add 0.1 ml of:
 - Purified water (cell 1);
 - Potassium cyanide solution (cell 2);
 - Test blood specimen (cell 3).
3. To each outer well add 0.5 ml of purified water and, on the opposite side of the outer well, 1.0 ml of dilute sulfuric acid.
4. Seal each well using silicone grease and carefully mix the components of the outer wells.
5. Incubate at room temperature for 20 minutes and then add 1 ml of aqueous methanol (1:1) to the centre wells.
6. Transfer the contents of the centre wells to 5.0-ml volumetric flasks and make up to volume with aqueous methanol (1:1).

Results

The red coloration obtained with cyanide-containing solutions is stable for about 15 minutes. Measure the absorbance of the solutions from cells 2 and 3 at 560 nm against the purified water blank (cell 1). Assess the cyanide ion concentration in the sample by comparison with the reading obtained from the standard.

Sensitivity

Cyanide, 0.5 mg/l

Toxicology

Treatments

Symptomatic management

Toxin specific measures

- Nitrite-thiosulfate, hydroxycobalamin

E) TOXICITY OF HOUSEHOLD PRODUCT

The ingestion of household products by a child is the most common pediatric medical emergency. Most household products are of relatively low toxicity. The top household products ingested are cleaning agents, cosmetics & personal products & berries. The manifestations when they do occur usually consist of mild GI upset.

Bleach is perhaps the substance most commonly involved in poisoning cases. When bleach is ingested orally it causes burning to the throat, mouth & esophagus. The tissue damage results in edema in the pharynx & larynx. In the stomach the presence of endogenous hydrochloric acid generates hypochlorous acid which is irritant & chlorine gas which may be inhaled causing toxic effects in the lungs.

II – MEDICAL TOXICANTS

Drugs are biologically active molecules used in the treatment, prevention & diagnosis of disease. However, drugs have made & will continue to make a major contribution to human health, we must accept the risks attached to these benefit.

The basic mechanisms for the toxicities arising from drugs are

- Direct & predictable toxic effects due to over doses
- Toxic effects occurring after repeated therapeutic doses



Toxicology

- Detection of bilirubin level and prothrombin time can tell prognosis
- Elevated aminotransferase levels can be seen
- Monitor blood glucose because in toxic cases hypoglycemia and hyperglycemia have been reported.
- The plasma creatinine rises more rapidly than BUN when renal failure is present. Liver failure may keep the BUN low.
- Serum amylase is determined because of reports of pancreatitis

Specific laboratory tests

Qualitative tests

Reagents (See annex I- number 8)

1. Saturated *o*-cresol.
2. Ammonium hydroxide, 4 mol/L
3. Concentrated hydrochloric acid.

Procedure

1. Mix 1 mL of specimen (victim or control urine, water blank) and 1 mL of concentrated hydrochloric acid. Heat at 100°C for 10 min.
2. Cool and add 100 μ L of the above solution to 10 mL of *o*-cresol reagent and then 2 mL of ammonium hydroxide, 4 mol/L

Result

Acetaminophen is hydrolyzed to *p*-aminophenol, which reacts with *o*-cresol and ammonium hydroxide to form an indophenol blue chromogen.

Quantitative tests

Principle

Acetaminophen and 3-acetamidophenol, added as an internal standard, are extracted from serum and analyzed by reverse-phase HPLC with an octadecylsilane bonded-phase column. The peak height absorbance ratio for acetaminophen relative to the internal standard is determined at 254 nm (annex II).

Reagents (see annex I)

1. Acetaminophen stock reference solution
2. Calibrator. Acetaminophen 20 and 100 g/mL.
3. 3-Acetamidophenol stock internal standard, 1000 mg/L.
4. 3-Acetaminophen working internal standard, 50 mg/ml.
5. Phosphate buffer.
6. Mobile phase: sodium acetate buffer/acetonitrile (92/8) by volume).

Procedure

1. Pipette 100 L of each calibrator, control, and victim's serum into properly labeled 13 x 100-mm glass tubes.
2. Add 100 L of working internal standard solution and 100 L of phosphate buffer (0.225 mol/L, pH 7.4). Mix.
3. Add 3mL of ethyl acetate. Mix in a Vortex mixer for 15 s.

saturable so the half life of aspirin increases significantly with only small increase in the number of tablets. The first sign of salicylate toxicity is often hyperventilation and respiratory alkalosis due to medullary stimulation. Metabolic acidosis follows due to accumulation of intracellular lactate as well as excretion of bicarbonate by the kidney to compensate for respiratory alkalosis.

Laboratory tests

Urine should be tested for pH, the presence of ketone bodies and hemoglobin. A rapid qualitative test for the presence of salicylates may be done in urine. Serum measurements of

Toxicology

salicylamide in urine, stomach contents or scene residues, first boil 1 ml of sample with 1 ml of aqueous hydrochloric acid (0.1 mol/l) for 10 minutes, & cool (filter if necessary), and then neutralize with 1 ml of aqueous sodium hydroxide (0.1 mol/l).

Results

A strong violet color indicates the presence of salicylates. Azide preservatives react strongly in this test, and weak false positives can be given by urine specimens containing high concentrations of ketone bodies.

This test is sensitive and will detect therapeutic dosage with salicylic acid, acetylsalicylic acid, 4-aminosalicylic acid, methyl salicylate and salicylamide.

Sensitivity

Salicylate, 10 mg/l

Quantitative assay

Specimen

Plasma or serum (1 ml)

Reagent

Trinder's reagent

Standards

Aqueous solutions containing salicylic acid at concentrations of 0, 200, 400 and 800 mg/l. Store at 4°C when not in use.

barbiturates (e.g. Phenobarbital) generally cause toxicity, but fatalities are more common with the latter. Mild intoxication resembles that of alcohol intoxication. Moderate intoxication is characterized by greater depression of mental status and severe intoxication causes coma.

Laboratory analysis

1. Plasma barbiturate (e.g. Phenobarbital) levels are helpful for making a diagnosis but of little value when predicting the severity of the over dose. $\mu\text{g/L}$.

Procedure

1. Add 5 ml of sample, 2 ml of hydrochloric acid and 60 ml of diethyl ether to a 250-ml separating funnel.
2. Lubricate (with purified water) and insert the funnel and shake gently for 2 minutes.
3. After standing for 5 minutes, and then discard the lower



12. Add 4 ml of filtrate from the test-tube to a clean, dry cell, add 50 μ l of concentrated ammonium hydroxide and mix using a plastic paddle. Check that the pH is about 10.
- 13.



Toxicology

Barbiturate, 2 mg/l

Treatment

GI decontamination

Alkalinization of urine

Hemodialysis

III. Environmental toxicants

Exposure of biological systems to chemicals may occur through environmental pollution of the atmosphere, water or soil. This results from industrial, agricultural & other human activities. Food born toxins derived from different microbes also can contribute in causing environmental intoxication. The atmosphere may be polluted by gases such as carbon monoxide & particulates.

a) Carbon monoxide poisoning

Carbon monoxide (CO) is a colorless, odorless gas that is ubiquitous because it is produced by the incomplete combustion of carbon compounds. The possibility of carbon monoxide poisoning is obvious for the victim of fire and smoke inhalation; but accidental and suicidal exposures are also common. The gas is readily absorbed across the alveolus and combines with hemoglobin with high affinity than oxygen. This displacement of oxygen from hemoglobin leads to a decrease in oxygen transport and causes tissue hypoxia. Elimination of carbon monoxide is predominantly through respiration; only about 1% is

metabolized to carbon dioxide. Victims with mild to moderate CO poisoning often complain of headache, dizziness and nausea and vomiting. Severe poisoning may result in chest pain,



Results

A pink tint in comparison with the colour obtained from a normal blood specimen suggests the presence of carboxyhaemoglobin.



Toxicology

remove all bound carbon monoxide (to give 0% HbCO). Again, take care to minimize frothing.

3. Add a small amount (about 20 mg) of sodium dithionite to each test solution (x, y and z) and also to 10 ml of ammonium hydroxide solution and mix well.
4. Measure the absorbance of solutions x, y and z against the dithionite-treated ammonium hydroxide solution at 540 nm and 579 nm.

Results

The percentage carboxyhemoglobin saturation (% HbCO) can be calculated from the equation:

$$\% \text{HbCO} = \frac{(A_{540}/A_{579} \text{ solution x}) - (A_{540}/A_{579} \text{ solution z})}{(A_{540}/A_{579} \text{ solution y}) - (A_{540}/A_{579} \text{ solution z})} \times 100$$

Approximate normal values are:

(A₅₄₀/A₅₇₉ solution y) = 1.5, corresponding to 100% HbCO

(A₅₄₀/A₅₇₉ solution z) = 1.1, corresponding to 0% HbCO.

Note that the hemoglobin content of blood varies from person to person, and thus the volume of diluents used may need to be altered. A dilution giving a maximum absorbance of about 1 absorbance unit at 540 nm is ideal.

N.B - It is important to use sodium dithionite that has been freshly obtained or stored in a sealed container in desiccators, since this compound is inactivated by prolonged contact with moist air.

- This method is unreliable in the presence of other pigments such



Toxicology

- CBC may reveal hemoconcentration (there will be increase in hematocrit level)
- Urinalysis shows a trace of protein
- Fecal leucocytes are absent in the presence of food-borne toxin induced illness
- Stool culture in general is not helpful for the diagnosis of microbial toxin intoxication. However, in a common source outbreak, examination of food, gastric contents, or stool may be useful.

Because the clinical course of poisoning with the following organisms is self-limited, preparing & identifying cultures is not cost-effective and rarely alters treatment plans. However, confirmatory tests may be warranted in the case of a mass outbreak of food poisoning. In such instances the following tests can be done.

1. *S. aureus* – Gram-stain of the suspected food and culture.
2. *B. cereus* - Bacteria count of 10^5 organisms per gram of food is suspicious for *B. cereus* because it is the normal fecal flora.
3. *C. perfringens* -anaerobic culture of the implicated foods can isolate *C. perfringens*.
4. *E. coli* - Stool culture identifies the presence of *E. coli* strains, but differentiation of the various strains of *E.coli* requires expensive animal testing, which is not cost effective

Toxin – specific tests

- Cholera – dark field microscopic examination of fresh-stool or stool culture on specialized selective culture media can be diagnostic.
- Food borne-botulism: stool, serum, vomits, gastric contents and suspected food should be collected to examine for spores or toxin.
- Hemorrhagic colitis caused by enterohemorrhagic E. coli can be detected by latex agglutination test.

Toxicology

department. Ethanol is mildly polar and readily penetrates cell membrane. Approximately 25% of ingested ethanol is absorbed unaltered from the stomach and the rest from the small intestine. Distribution is rapid and wide. Over 90% of alcohol consumed is oxidized in the liver; much of the remainder is excreted through the lungs and in the urine. Alcohol is a central nervous system depressant. It can cause sedation, impaired motor function, slurred speech, emesis, ataxia etc. At high blood concentrations, it induces coma, respiratory depression, and death.

Laboratory analysis

Routine laboratory tests

CBC, electrolyte, BUN, glucose, creatinine, arterial blood gas analysis

Specific laboratory tests

Qualitative test

Specimen

Urine, stomach contents, scene residues.

Reagent

Potassium dichromate (25 g/l) in aqueous sulfuric acid (500 ml/l)

Procedure

1. Apply 50 μ l of potassium dichromate solution to a strip of glass- fibre filter- paper and insert the paper in the neck of a test- tube containing 1 ml of sample.



fluoride has been added. These solutions are stable for up to 1 month if stored at 4°C in well-sealed containers.

Procedure

1. Add 0.5 ml of blood to 2 ml of perchloric acid solution in a test-tube.
2. Vortex-mix for 30 seconds and then centrifuge for 5 minutes.
3. Add 0.1 ml of the supernatant (or 0.2 ml of an aqueous dilution (1:9) of plasma/serum) to a 10-ml tube containing 4.5 ml of semicarbazide reagent and vortex-mix for 10 seconds.
4. Add 0.1 ml of NAD solution and 0.02 ml of ADH suspension and mix gently so as not to cause foaming.
5. Allow to stand for 70 minutes at 20-25°C and measure the absorbance at 340 nm against a reagent blank

Results

Construct a calibration graph of absorbance against blood ethanol

Treatment

Symptomatic management



Laboratory analysis

General test

CBC (polymorph nuclear leukocytosis), electrolytes, BUN, creatinine, arterial blood-gas analysis, liver function tests, urine analysis (Glycosuria)

Toxin specific tests

- Serum nicotine levels should be determined as early as possible, but the short half-life of nicotine makes it difficult to accurately assess the level of intoxication.
- Urine nicotine levels – are inconsistent owing to the altered excretion of nicotine with changes in urine pH. They may be useful as a guide to the level of chronic exposures.

C. Opioids

Opioids comprise a broad spectrum of substances that include opiate alkaloids (e .g morphine & codeine), synthetic opioids (e .g pethidine) & semi synthetic opioids (e .g heroin). They exert their

are below 2000 ng/ml (the detection cutoff sensitivity of the test) unbound antibody-dye conjugate binds to immobilized antigen conjugate in the Test Zone ("T"), producing a pink-rose colored band that indicates a negative result. Conversely, when opiates levels are above the detection limit, antibody-dye conjugate binds to the free drug, forming an antigen-antibody-dye complex. The complex competes with immobilized antigen conjugate in the Test Zone, preventing the development of a pink-rose colored band. Regardless of the test result, a color band is produced in the Control Zone ("C") by a non-specific sandwich dye conjugate reaction. This band serves as a built-in quality

Specimen

Urine

Procedure

1. Collect a urine sample from test subject using a suitable clean container preferably glass
2. Refrigerated specimens or other materials should be equilibrated to room temperature before testing
3. Open the foil pouch at the notch, remove the test device, and label the device with specimen ID.
4. Holding the dropper vertically, add four drops of urine into the

specimen. To confirm negative results, a complete reaction time of 8-10 minutes is required. Do not interpret results after 15 minutes.

Results

Positive: One pink rose band appears in the control zone and no band appears in the test zone. A positive result indicates the opiates level is 2000ng/ml or higher in the test urine sample.

Negative: One band appears in the test zone and other band appears in the control zone. A negative result indicates that the opiates level is below the detection sensitivity of 2000ng/ml.

N.B. any line, no matter how faint appearing in the test area confirms a negative test.

Invalid: If there are no distinct color bands visible in both the test zone and the control zone or if there is a visible band in the test zone but not in the control zone, then the test is invalid. In the instant, retesting of the specimen is recommended.

V. Natural toxicants

Natural substances are also still occasionally featured in accidental poisoning cases, when compared to poisoning by others. 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. Natural toxins are of diverse structure

a. Animal toxins

Animal toxins comprise a diverse range of structures & modes of



met with little success. A radioimmunoassay was developed for this purpose, but it has never become a practical



Toxicology

5. 5. What are animal & plant toxicants? What kind of routine laboratory tests are used in snake bite?

GLOSSARY

Abuse - Excessive or improper use of drugs or other substances.

Acetylcholine - the main neurotransmitter of the vertebrate and invertebrate in the peripheral nervous systems

Acetylcholinesterase - enzyme that hydrolyses the neurotransmitter acetylcholine.

Acidosis - Pathological condition resulting from accumulation of acid in, or loss of base from, the blood or body tissues.

Acute - Sudden or short-term.

Anemia - Deficiency of erythrocytes or of hemoglobin in the blood.

Antibody - A protein produced in the body in response to exposure to an antigen; it recognizes and specifically binds the antigen.

Anticoagulant - A drug that prevents clotting of blood.

Antidote - An agent that neutralizes or opposes the action of a poison on an organism.

Arrhythmia (dysrhythmia) -Any variation from the normal rhythm of the heartbeat.

Antigen -Any substance that stimulates the body to produce an antibody.

Bilirubin -A pigment, derived from the breakdown of hemoglobin that occurs in soluble form in blood and in bile.

Blank- Used in analytical chemistry to denote a specimen not containing the analyte of interest and from which a background reading can be obtained.

Carboxyhaemoglobin - Product formed when carbon monoxide binds to hemoglobin.

Chelate - Compound in which a central metallic ion is attached

Toxicology

Corrosive - Able to eat away or dissolve by chemical action.

Cosmetic - Concerned with improving appearance or hygiene.

Cross-contamination - Accidental introduction of an impurity.

Crystalluria - Presence of crystals in the urine.

Cutaneous - Associated with the skin.

Cyanosis - Blue appearance, especially of the skin and mucous membranes, due to deficient oxygenation.

Dermal - Relating to the skin.

Detergent - A chemical cleaning agent.

Diuresis - Increased production of urine.

Diuresis, forced - Abnormally enhanced urine production, for example following administration of intravenous fluids or diuretics.

Drug - A substance that, when administered to an organism or a system derived from an organism, may modify one or more of its functions.

Emesis - Vomiting.

Emetic - Substance causing emesis.

Erythrocyte - Red blood cell.

Fumigant - A vapour used to kill pests.

Fungicide - A pesticide used to kill fungi or check the growth of spores.

Haematocrit - Erythrocyte volume fraction; the ratio by volume of the blood cells to plasma.

Haematuria - presence of red blood cells in the urine.

Haemodialysis - Procedure whereby blood is dialysed against a large volume of isotonic fluid outside the body and then returned to the systemic circulation. Used to remove unwanted compounds of low relative molecular mass from the circulation.



Pesticide -Substance used to kill or control any pest, including animals, plants, fungi, or other organisms in agricultural, industrial and domestic situations

Pipette, automatic -Device used to dispense repeatedly known volumes of a fluid.

Pipette, positive-displacement- Device with a washable tip, used to take up and dispense known volumes of a fluid, and in which the plunger is in contact with the fluid. Used to dispense viscous solutions such as whole blood.

Pipette, semi-automatic- Device, often with disposable tips, used to take up and dispense known volumes of aqueous fluids such

Toxicology

other procedures requiring efficient mixing of relatively small quantities of material (up to about 10 ml total volume).

Xenobiotic- Compound foreign to the biotransformation of an organism.

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ANNEX I

Preparation of reagents

1. Reagents for lead qualitative tests

- a. Sodium tartrate buffer, pH 2.8.

Sodium bitartate.....19 g

Tartaric acid.....15 g

Purified water.....1000ml

PH.....2.8

- b. Aqueous sodium rhodizonate solution (10 g/l).

2. Reagents preparation for quantitative tests of lead using AAS

- a.

10, 20, 40, and 60 g/dL, which are used to calibrate the instrument as described below.

- d. Controls- The control material is a commercial whole blood control.

3. Thin layer preparation for organophosphorus insecticides

- A. Reconstitute the extract in 100 μ l of methanol and spot 20 μ l on a column marked on the plate.
- B. Spot 10 μ l of the standard mixture on a second column.
- C. Develop the chromatogram (10-cm run) using

cyclohexane:

oe 0 Tc 10 0 0 15 Tm (A.) TJET Q q 1 0 0 -1 0

B. Ammonium hydroxid, 4 mol /L - Dilute 284 mL of concentrated ammonium hydroxide to 1 L with deionized



- F. Mobile phase: sodium acetate buffer/acetonitrile (92/8) by volume) - Add 80 mL of acetonitrile to a 1-L volumetric flask and dilute to volume with 0.01 mol/L sodium acetate buffer, pH 4.0. Filter through a 0.5- μ m pore-size filter and degas by applying gentle vacuum

9. Reagents for quantitative test of ethanol.

A. Semicarbazide reagent.

Tetrasodium pyrophosphate dehydrated10 g.

Semicarbazide hydrochloride.....2.5 g

Glycine.....0.5 g

Distilled water.....250 ml

Sodium hydroxide (2 mol/l)10ml

Dilute the solution by Distilled water up to 300ml

- B. Aqueous nicotinamide adenine dinucleotide (NAD) This solution is stable for 2-3 months at 4°C, but can be decomposed by vigorous agitation.

C.

ANNEX II

Apparatuses

1. Atomic Absorption spectrophotometer for quantitative analysis of Lead



acetaminophen concentrations of 0 to 200 or 200 to 500- g/mL, respectively. The pump flow rate is 1.5 ml/min.

4. Summary of basic equipment required for toxicological analyses

Reliable, regularly serviced and calibrated laboratory balances

Bench-top centrifuge (electrical or hand-driven) for

Toxicology

Conway microdiffusion apparatus

Porcelain spotting tile

Modified Gutzeit apparatus



Example of an analytical toxicology request form

	Date or admission :	
	Date / time of ingestion or exposure:	
	Drugs prescribed or used in treatment	
Doctor: Telephone: Hospital address for report: Signed: _____ Date: _____		

Toxicology

B l o o d (1 0 m l			priority:
heparinized)			
Urine(50ml)			
Other(give details)			



