LECTURE NOTES

For Medical Laboratory Science Students

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Toxicology

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PREFACE

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. Toxicology can be an independent descriptive, empiric discipline to the fact of difficulty in diagnosis, controversial management and unknown end points. Many lethal exposures deserve early diagnosis & management before the confirmatory evidences. This lecture note on toxicology is primarily inspired for undergraduate laboratory technology students who participate in the care of poisoned patients. However, other health professionals whose carriers involve related aspects can find it relevant. The outline format of the lecture note allows for particular rapid review of essential information.

The first two chapters of this lecture note focus on the introduction, basic concepts of toxicology and general approach to poisoned patients. These chapters empower the basic toxicological backgrounds. The third, fourth & fifth chapters are concerning the basic toxicological testing methods, which planned specifically as a subject matter to the

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 revise the references. At last, we hope this material is valuable in the handling &management of poisoned patients by health professionals

The authors
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ABBREVATIONS

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

INTRODUCTION TO TOXICOLOGY

Learning Objectives

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

1. Understand the history of toxicology, &epidemiology of poisoning.
2. Define different terminologies used in toxicology.
3. Understand the basic classification of toxicology.
4. Describe toxicokinetics & toxicodynamics.
5. Describe the potential causes of toxicity
6. Understand the environmental consideration of toxicology.
7. Describe poison prevention & control strategies.

INTRODUCTION

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. This short chapter presents an overview of the broad topic of toxicology.

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

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

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 ***synergistic, additive, Potentiation & antagonistic***

Synergism-when the effect of two chemicals is greater than the effect of individual chemicals e .g carbontetrachloride + alcohol= more toxic to the liver than the sum of the individual drugs

Additive effect- when the total pharmacological action of two or more chemicals taken together is equivalent to the summation of their individual pharmacological action.

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 -is the phenomenon of opposing actions of two chemicals on the same system e .g paracetamol causes serious liver damage when given with alcohol or barbiturates, both of which induce drug metabolizing enzymes.

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 in USA.

- Environmental protection agency regulates pesticides, toxic chemicals, hazardous wastes and toxic pollutants in USA
- Occupational safety and health administration regulates the safe conditions for employees in USA
- Drug administration & control authority (DACA) - regulates drugs, cosmetics and medical devices &supplies in Ethiopia.

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 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 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:

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

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

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.

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

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

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

N.B - 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

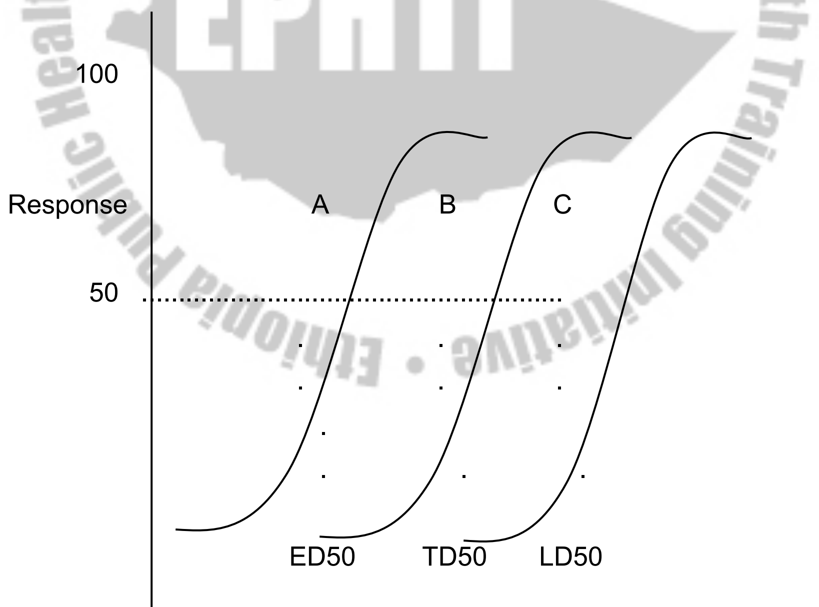
The general dose-response principles are of crucial importance in determining the severity of the intoxication. We have two types of responses so called *Quantal dose response* (all- or – none response) & *graded dose response* (when dose increases, the response increases in graded fashion). Both responses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

parameters that are derived from the dose response relationships are

Median lethal dose (LD50) – 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.



Dosage

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

a. 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
b. Depress or stimulate the CNS, producing coma (sedative-hypnotics) or convulsions (Sympathomimetics such as cocaine, amphetamines)
c. Affect the autonomic nervous system, producing cholinergic action (organophosphate insecticide)
d. Affect the lungs by aspiration (hydrocarbon)
e. Affect the heart and vasculature producing myocardial dysfunction, dysrrhythmias (antiarrhythmic agents) and hypertension or hypotension
f. Produce local damage (caustics and corrosives)
g. 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.

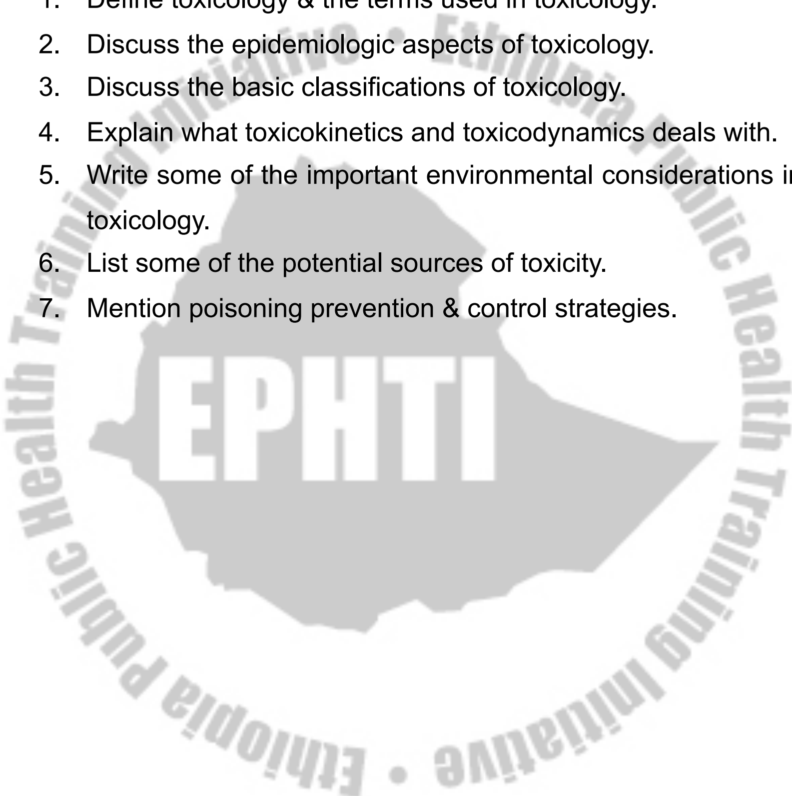
In ecotoxicology there are three interacting components; the ***toxicant,*** the ***environment*** and the ***organisms*** (community, population or ecosystem).

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 hunts 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 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

GENERAL APPROACH TO A POISONED VICTIM

Learning Objectives

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

1. Understand diagnosis of poisoning by history, physical examination and different investigations
2. Understand the basic principles of management of poisoning

Introduction

The purpose of this chapter is to provide guidelines for evaluating the severity of an exposure to a potentially toxic substance, clues to the identity of the offending substance (its clinical effects on vital functions, its odor, and its effect on the skin), and, most importantly, how to manage the severely intoxicated victim initially. The trained analyst can play a useful role in the management of victims poisoned with drugs or other chemicals. However, optimal analytical performance is only possible when the clinical aspects of the diagnosis and treatment of such victims are understood. The analyst must therefore have a basic knowledge of emergency medicine and

intensive care, and must be able to communicate with clinicians. In addition, a good understanding of pharmacology and toxicology and some knowledge of active elimination procedures and the use of antidotes are desirable. This chapter aims to provide some of the basic information required in the general approach of poisoned victims.

General approach to a poisoned victim

Diagnosis of poisoning may be difficult. The victim may either be unconscious or may not admit self-poisoning. Therefore, a suspicious mind is required. When acute poisoning is suspected, the clinician needs to ask a number of questions in order to establish a diagnosis (history of present illness). In the case of an unconscious (comatose) victim, the circumstances in which the victim was found and whether any tablet, bottles or other containers (scene residues) were present can be important. If the victim is awake, he or she should be questioned about the presence of poisons in the home or workplace. The victim's past medical history (including drugs prescribed and any psychiatric illness), occupation and hobbies may also be relevant, since they may indicate possible access to specific poisons.

Physical examination of the victim may indicate

- The poison or class of poison involved.
- The clinical features associated with some common poisons may be specific. For example, the combination of pin-point pupils, hyper salivation, incontinence and respiratory depression suggests poisoning with a cholinesterase inhibitor such as an organophosphorus pesticide. However, the value of this approach is limited

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.
- Finally, poisoning with certain compounds may be misdiagnosed, especially if the victim presents in the later stages of the episode. Examples include: cardiorespiratory arrest (cyanide), hepatitis (paracetamol) and so on.

N.B Generally Physical examination should include

- – Vital signs
- – Evaluation of specific parts of the body

Investigations

a) General laboratory tests
- Hematological
- Biochemical

b) Toxicological studies

c) Electrocardiogram

d) X-ray findings

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).

- 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 summery 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.

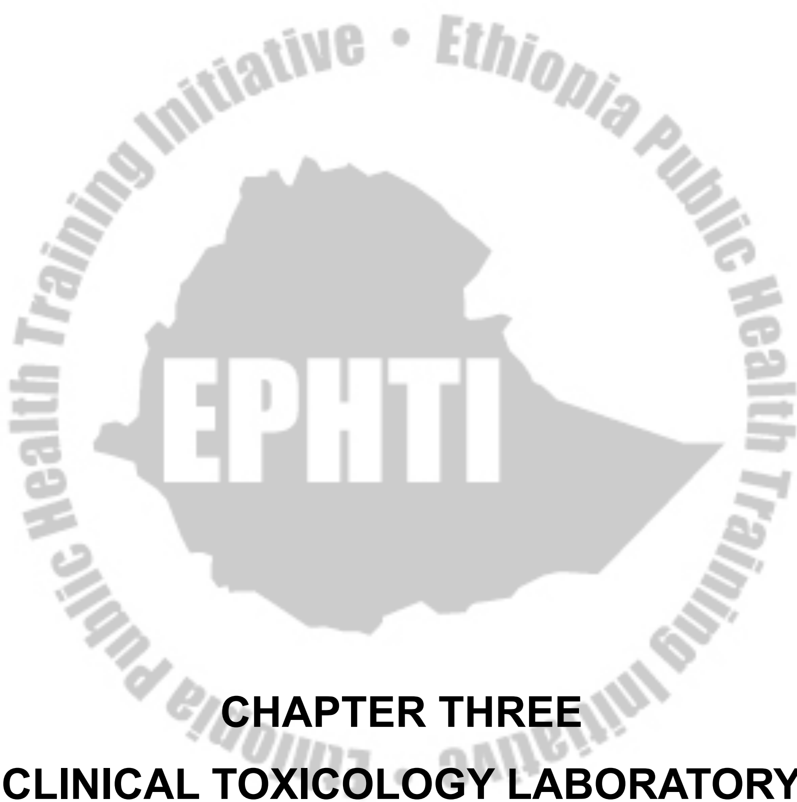
Some antidotes &protective agents used to treat acute poisoning

Antidote	Indication
• Acetylcysteine	Paracetamol

- Atropine Organophosphate
- Deferoxamine Iron
- Methylene blue Nitrates
- Physiostigmine Atropine
- Naloxone Opioids
- Pyridoxine 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 able to:

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

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

Toxicology

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. 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 (detailed practical aspects of analytical toxicology is discussed in the next chapters).

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 (purity or cleanliness) 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 (see annex II).

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 (See annex I).

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^{-} + HCO_3). 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.

Osmolal gap can be calculated:

Osmolsl gap (Osmolarity) = $2(Na^+)$ + Glucose ÷18 + BUN ÷ 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

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 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 properly, the laboratory technician should have a rudimentary familiarity with the analytic methods employed. Several methods exist, varying in sensitivity, specificity, assay time, and cost. The choice depends on the size and budget of the institution, the types of victims served the proximity to more elaborate toxicology facilities, and other factors. This chapter focuses on the practical aspects of analytical toxicology.

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.
b. The toxicology screen is performed on a specimen collected at a time **outside the detection period** for a particular toxin. Most of the common drugs of abuse are detectable in urine for 48-72 hours after ingestion, e.g., opiates
c. The toxins may be present in concentrations **below the limits of detectability** for the method used.

In general, when faced with an unresponsive victim and an incomplete clinical history, minimum testing should include quantification of **ethanol, acetaminophen, salicylate, and barbiturates.**

II. Confirmatory methods

Confirmatory methods are designed to have near-perfect specificity and tend to be technically much more complex and demanding. Confirmatory methods are of relatively little importance in the context of emergency toxicology. Their principal use is in legal situations, where it must be established beyond a reasonable doubt that a particular drug was present. In these cases, integrity of specimen handling (chain of custody) becomes

as important as the analytic procedure itself in order to rule out the possibility of specimen tampering or substitution.

B. 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.

- **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. **Nuclear magnetic resonance** (NMR)

NMR is another technique that can perform both definitive substance identification and screening of body fluid, although at a much lower sensitivity than GC-MS. The technique, however, is even more expensive than GC-MS.

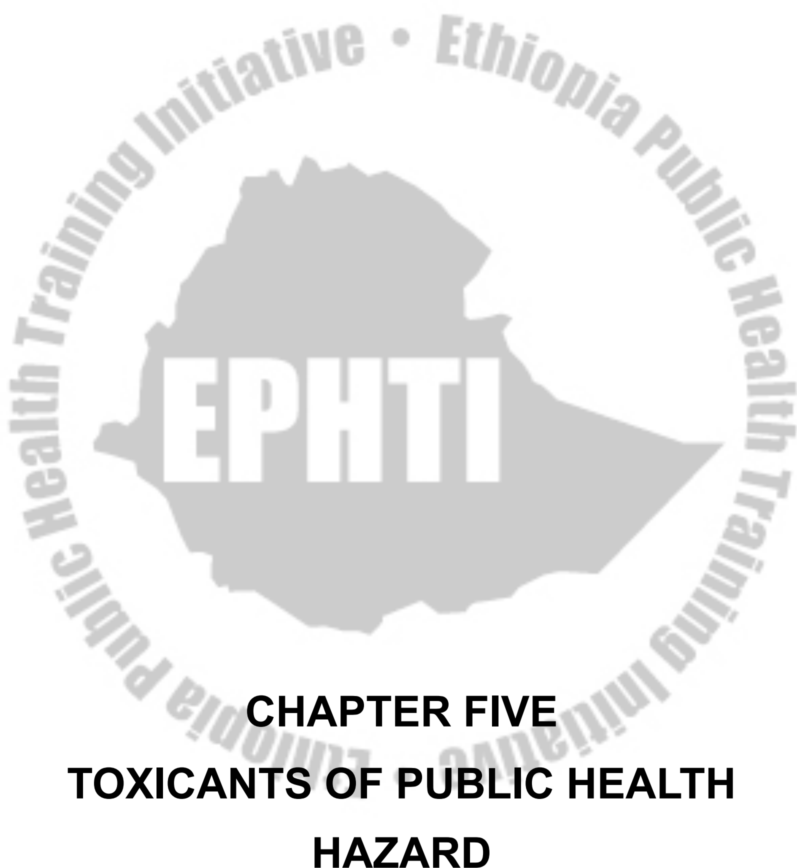
VII. **Atomic absorption, plasma emission, neutron activation, and x-ray fluorescence**

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.**

Exercise

1. What is the test methods used in practical aspects of analytical toxicology?
2. Explain the common analytical toxicology techniques.



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 toxicological laboratory investigations.
2. Describe medical poisoning caused by acetaminophen, salicylates & barbiturates with their toxicological laboratory investigations.
3. Explain the environmental toxins like carbon monoxide, & food born toxins with their toxicological laboratory investigations.
4. Understand the common drugs of abuse like alcohol, nicotine, & opioids with their toxicological laboratory investigation.
5. Describe animal intoxications like snakebite with their laboratory investigation.

Introduction

The rapid industrialization and successful green revolution have introduced a large variety of chemicals into our environment. The species and varieties of environmental chemicals are as many as we can visualize. We may however, characterize them as: industrial chemicals which include organic and inorganic substances, metals, gases, fumes, solvents, and intermediates; agrochemicals, a major input of farming industry, comprising a variety of pesticides, fertilizers and growth promoters; pharmaceuticals, in innumerable number; and food additives, plastics, cosmetics etc. These have caused a great danger and put human and environment at a high risk. This chapter is meant for discussion of some of the important toxicants of public health hazard.

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...

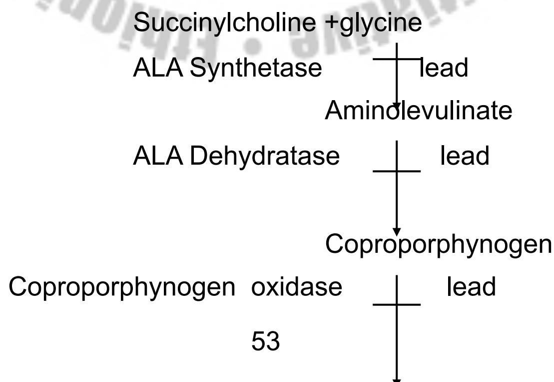
Lead Poisoning

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

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 2-3weeks 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).



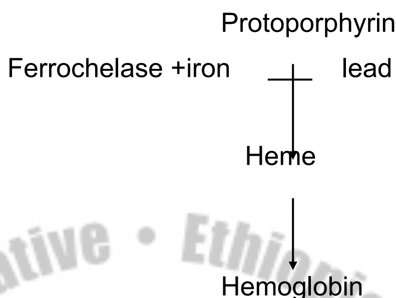


Fig. Lead interference with the biosynthesis of heme

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)

Laboratory findings

A. Complete blood count

- Anemia –- Hemoglobin level of less than 10gm/dl can be seen
- Reticulocytosis – results from early release of immature RBCs. It is not present in iron deficiency anemia so it is valuable for differentiating the two forms of anemia.
- Eosinophilia – common finding but non specific
- Basophilic stippling of erythrocytes on wright stain of peripheral blood has been observed to be a less frequent occurrence than anemia & the finding is less non specific.

B) Serum Lead level

Levels of 30-60μg/dl are regarded as significant for lead toxicity. Atomic absorption spectrometry (AAS) is the most

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 ($CaNa_2$-EDTA) provocation test

CaNa2-EDTA administered to evaluate the chelatable lead store.

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 µl of acidified solution on to phase-separating filter-paper and add 50 µl 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 chloride concentration is to be avoided, because the chloride salt of lead is volatile at the charring temperatures used in atomic absorption analysis (Annex II).

Reagents (see annex I)

1. Ultra pure concentrated nitric acid.
2. Diluent (matrix modifier)
3. Calibrators
4. Controls. Commercial whole blood control.

Procedure

1. The first two specimens analyzed represent blanks. They consist of 0.25 mL of triple-distilled water and 1.0 mL of diluent.
2. Calibrating solutions are made by mixing 1.0 mL of the diluent with 0.25 mL of each calibrator.
3. The control pool and victim specimens are prepared for analysis by mixing 0.25 mL of each sample with 1.0 mL of diluent.
4. Add 10 μL of matrix modifier to 10μL of each sample and inject into the L'vov platform.

5. Record the absorbance peak area generated by each specimen. Repeat the analysis at an appropriate dilution for any specimen with an absorbance greater than that of the high concentration calibrator

Calculation

1. Subtract the blank absorbance peak area from each specimen absorbance peak area.
2. Perform a best-fit regression analysis of the calibrator concentrations versus the respective absorbance peak area to define the calibration curve.

- Compare the absorbance peak area derived from each specimen against the calibration curve to determine the concentration of lead. If the results among duplicates vary by more than 10%, sample contamination during processing is likely to have occurred.

Management

- Chelating agents (Antidotes) E.g. dimercaprol, CaNa2-EDTA
- Symptomatic management

B. HYDROCARBON POISONING

Hydrocarbon comprises a broad group of organic compounds that contain hydrogen & carbon atoms only. Most, but not all, are derived from petroleum distillation. Hydrocarbons are found alone or incombination with others in a wide variety of commercial products ubiquitous around the home or work place. Lighter fluid, paint thinners, &removers, some furniture polishes, cleaning agents, solvents, various automotive products &

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.
- Arterial blood gas analysis (ABGs) must be measured in all victims with respiratory symptoms. Varying degrees of hypoxia without hypercarbia are the most common finding.
- Occasionally a metabolic acidosis is seen.
- The CBC may show leukocytosis
- Blood hydrocarbon levels are not readily available &have little diagnostic, prognostic, or therapeutic value. Qualitative testing is occasionally needed for forensic purposes.

Specific tests

Qualitative test

Reagents

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.

Organophosphurus insecticides

These agents are utilized to combat a large variety of pests. Some of these agents are used in human and veterinary medicine as local or systemic antiparasitics or in circumstances in which prolonged inhibition of cholinesterase is indicated. The

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 organophosphate poisoning and parallels the activity of neurosynaptic cholinesterase
- Plasma cholinesterase levels are a less specific but more sensitive measure of toxicity

Specific tests

Qualitative test

Specimen

Stomach contents, scene residues.

Reagents

1. Sodium bicarbonate (solid).

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.
3. Allow to stand for 5 minutes, take off the upper, ether layer and re-extract with a second 5-ml portion of methyl tertiary-butyl ether.
4. Combine the extracts, filter through phase-separating filter- paper into a clean tube and evaporate to dryness under a stream of compressed air or nitrogen.
5. Analyze the final solution in thin layer chromatography (see annex I-number 3)

Results

The compounds of interest give purple spots on a pale brown background.

Sensitivity

Organophosphorus pesticide, 5 mg/l

Confirmatory test

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 µl of control plasma to one tube and 20 µl of test plasma to a second.
3. Add 20 µl of pralidoxime solution and 20 µl 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 atopine, 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 & affect brain cholinesterase activity

Sign & symptoms may be more rapid &usually abate within 24hrs regardless of therapy

Laboratory findings

Toxicology

- 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)
2. Furfuraldehyde solution (100 ml/l) in methanol, freshly prepared.
3. Concentrated hydrochloric acid (relative density 1.18).

Procedure

1. Acidify 1 ml of sample with 0.5 ml of dilute hydrochloric acid and extract with 4 ml of chloroform on a rotary mixer for 5 minutes.
2. Centrifuge for 5 minutes, discard the upper, aqueous layer and filter the chloroform extract through phase-separating filter-paper into a clean tube.
3. Evaporate the extract to dryness under a stream of compressed air or nitrogen at 40°C.
4. Dissolve the residue in 0.1 ml of methanol, apply a spot of the solution to filter-paper and allow drying.
5. Apply 0.1 ml of furfuraldehyde solution to the spot;
6. Allow drying and exposing the paper to concentrated hydrochloric acid fumes for 5 minutes in a fume cupboard.

Results

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 poisoning may not be evident until a few days after exposure.

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 20µg/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 been also shown to bind to many other proteins. Cyanide sources can be certain plants (E.g. cassava), cyanide gas (e.g. cigarette smoke), medicines that liberate cyanide (E.g. Nitroprusside), exposure in fires etc...Oral absorption of cyanide is rapid and the toxic effects can present within minutes. It is widely distribution in the body & about 80% eliminated through the kidney in a form of thiocyanate. Organs such as the brain &heart are affected particularly as they require ATP &have limited capacity to cope with a deficit. The heart has only enough ATP to last 3 minutes &so cyanide may cause death as a result of heart or respiratory failure. The sign and symptoms of cyanide poisoning include headache, hypoxic convulsion, and respiratory distress, cyanide odor…

Laboratory analysis

i) General tests

1. Chemistry Tests

- Because of the blockade of aerobic biotransformation, cyanide produces an anion gap metabolic acidosis secondary to the production of lactic acid
- Glucose catabolism is also altered, and the blood glucose level may be elevated

2. Blood gas analysis

- PaO_2 and oxygen saturation are unaltered except in severe cases where respiratory failure occurs.
- pH and $PaCO_2$ are altered in different ways, depending on the severity of poisoning

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

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**

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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

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

- Direct but unpredictable toxic effects occurring after single therapeutic doses due to idiosyncratic response (peculiar response of an individual to a drug).
- Toxic effects due to another drug or substance interfering with the disposition or pharmacological response.

So, we do have a short overview of some of the common medical toxicants

A. Acetaminophen

Acetaminophen is analgesics for mild &moderate pain which is very safe provided only the normal therapeutic dose. Acetaminophen is one of the drugs most commonly involved in suicide and accidental poisoning. Initial symptoms after an overdose are mild and non specific, often resulting in delayed arrival for medical care or a missed diagnosis. Acute ingestion of more than 150-200mg/kg (children) or 7gm (adults) is considered potentially toxic. Paracetamol is metabolized mainly by conjugation & minor proportion metabolized by oxidation which produces toxic products which detoxified normally. However, overdoses change the metabolic scheme giving a rise in toxic metabolite which react with liver proteins & cause tissue damage (leading to hepatic toxicity). Initially, the victim is asymptomatic or has mild GI upset (nausea, vomiting) which is followed by evidence of liver injury.

Laboratory analysis

- Severity of poisoning is determined from serum acetaminophen level.

- 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 100^0C for 10 min.
2. Cool and add 100 μL 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 μg/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 μL of each calibrator, control, and victim's serum into properly labeled 13 x 100-mm glass tubes.
2. Add 100 μL of working internal standard solution and 100 μL 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.

4. Centrifuge all tubes at 1000 x *g* for 5 min.
5. Transfer the organic (top) layer to labeled 12 x 75-mm glass tubes and evaporate under a stream of dry air at 50 ^{0}C.
6. Reconstitute the contents of each tube with 100 μL of the mobile phase and inject 10 to 20 μL into the HPLC instrument.

Calculation

Determine the peak height (or peak area) ratios of acetaminophen relative to the internal standard. Calculate the concentration of acetaminophen in the unknown by comparing its peak height ratio versus acetaminophen concentration response for the calibrator.

Treatment

- GI decontamination
- Antidote (acetylcysteine)

B. Aspirin (salicylate)

Acetylsalicylic acid, commonly known as aspirin, is still one of the most widely used minor analgesics. Salicylate poisoning is a much less common cause of childhood poisoning deaths since the introduction of child-resistant container and the reduced use of baby aspirin. Salicylates, however still accounts for numerous suicidal and accidental poisonings. Salicylate Poisoning can also result from chronic over medication; this occurs most commonly in elderly victims using salicylates for chronic pain because of impaired biotransformation, excretion & others. Salicylic acid is then metabolized by conjugation. These conjugation steps are

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 salicylate are important after acute ingestions.

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Specific laboratory tests

Qualitative test

Specimen

Urine, stomach contents, scene residues.

Reagent (see annex I)

Trinder's reagent

Procedure

Add 0.1 ml of Trinder's reagent to 2 ml of sample and mix for 5 seconds.

N.B- To test for acetylsalicylic acid or methyl salicylate in stomach contents or scene residues, and to test for

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.

Procedure

1. Add 5 ml of Trinder's reagent to 1 ml of sample or standard.
2. Vortex-mix for 30 seconds and centrifuge for 5 minutes.
3. Measure the absorbance of the supernatant at 540 nm against plasma blank

Results

Calculate the plasma salicylate concentration from the graph obtained on analysis of the salicylate standards. Some salicylate metabolites interfere, but plasma concentrations of these compounds are usually low. Oxalates, for example, from fluoride/ oxalate blood tubes, also interfere in this test.

Sensitivity

Salicylate, 50 mg/l

Treatment

- GI decontamination
- Facilitating diuresis
- Symptomatic management

C) Barbiturates

Barbiturates belong to a class of sedative-hypnotic drugs with abuse potential & a recognized withdrawal syndrome. Toxic manifestations of barbiturates vary with the amount of ingestion, type of drug and time elapsed since ingestion. Lower doses of short acting barbiturates (E.g. pentobarbital) than the long-acting

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.
2. As with alcohol, chronic abusers of Phenobarbital may have elevated serum levels with little CNS depression.

Specific laboratory tests

Quantitative assay

Specimen

Whole blood, plasma or serum (5 ml)

Reagents (see annex I)

1. Borate buffer, pH 8.4.
2. Aqueous hydrochloric acid (2 mol/l)
3. Concentrated sulfuric acid (relative density 1.83).
4. Concentrated ammonium hydroxide (relative density 0.88).
5. Sodium sulfate/charcoal mixture.

Standards (see annex I)

Solutions containing barbital at concentrations of 5, 10, 25 and 50 mg/l in blank human plasma

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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 aqueous phase, add the diethyl ether extract to 10 ml of borate buffer in a second separating funnel and mix for 1 minute.
4. Allow to stand for 5 minutes and again discard the lower, aqueous phase through the funnel tap.
5. Wash round the funnel with 5 ml of purified water; allow standing for 5 minutes and again discarding the lower, aqueous phase through the funnel tap.
6. Add about 4 g of sodium sulfate/charcoal mixture to the ether extract in the funnel, shake to disperse, and filter the extract through phase-separating filter-paper into a 150-ml conical flask.
7. Add a further 20 ml of diethyl ether to the separating funnel, shake and add to the extract in the flask through the filter funnel.
8. Evaporate the extract to dryness on a water-bath at 40°C under a stream of compressed air or nitrogen.
9. Add 5.0 ml of purified water to the dry extract in the flask, swirl gently and allow to stand for 5 minutes.
10. Filter the reconstituted extract through phase-separating filter-paper into a 12.5-cm test-tube.
11. Check the spectrophotometer zero at 240 nm using purified water in both sample and reference positions.

12. Add 4 ml of filtrate from the test-tube to a clean, dry cell, add 50 µl of concentrated ammonium hydroxide and mix using a plastic paddle. Check that the pH is about 10.
13. Quickly measure the absorbance at 240 nm against purified water blank. If necessary, accurately dilute a portion of the extract with purified water to bring the reading on to the scale, and record the magnitude of the dilution. If a scanning spectrophotometer is available, scan in the region 200-450 nm.
14. Repeat the reading or scan after 5 minutes.
15. Add 0.1 ml of concentrated sulfuric acid to the cell, mix using the plastic paddle, and check that the pH is about 2.
16. Repeat the reading (240 nm) or scan (200-450 nm).

Results

1) To perform a quantitative measurement, measure the difference between absorbance at pH 10 and at pH 2, construct a calibration graph by analysis of the standard barbiturate solutions, and calculate the barbiturate concentration in the sample.

   Alternatively, use the following formula:

   ((absorbance at pH 10) - (absorbance at pH 2)) × Dilution factor (if any) × 25 = barbiturate (mg/l)
2) Sample volumes of less than 5 ml may be used, but there will be a corresponding loss of sensitivity unless "micro"-volume fused silica spectrophotometer cells are available.

Sensitivity

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, dyspnea, syncope, seizures and coma.

Laboratory analysis

- Carboxyheamoglobin level – should be measured as early as possible to establish the diagnosis of carbon monoxide poisoning. Carboxyheamoglobin should be measured from blood sample using spectrophotometric methods. Although not as accurate, carboxyheamoglobin levels can be estimated from expired air using a breath analyzer for carbon monoxide.
- Arterial blood gas assays are poor indicators of carbon monoxide poisoning. The PaO_2 is often normal, as it is a measure of oxygen dissolved in plasma, not a measure of oxygen bound to hemoglobin.

Specific laboratory tests

Qualitative test

Applicable to whole blood treated with heparin, edetic acid or fluoride/oxalate.

Reagent

Aqueous ammonium hydroxide (0.01 mol/l)

Procedure

Add 0.1 ml of blood to 2 ml of ammonium hydroxide solution and vortex-mix for 5 seconds.

Results

A pink tint in comparison with the colour obtained from a normal blood specimen suggests the presence of carboxyhaemoglobin. Cyanide may give a similar tint, but acute cyanide poisoning is generally much less common than carbon monoxide poisoning.

Sensitivity

HbCO, 20%

Quantitative assay

Specimen:whole blood treated with heparin, edetic acid or fluoride/ oxalate.

Reagents

1. Aqueous ammonium hydroxide (1 ml/l).
2. Sodium dithionite (solid, stored in a desiccator).
3. A supply of pure carbon monoxide or carbon monoxide/nitrogen.
4. A supply of oxygen or compressed air.

Procedure

1. Add 0.2 ml of blood to 25 ml of ammonium hydroxide solution and mix.
2. Take three approximately equal portions: x, y and z. Keep portion x in a stoppered tube while the following procedures are performed:

   (a) Saturate portion y with carbon monoxide (to give 100% HbCO) by bubbling the gas through the solution for 5-10 minutes. Take care to minimize frothing.

   (b) Saturate portion z with oxygen by bubbling pure oxygen or compressed air through the solution for at least 10 minutes to

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:

$$\%HbCO = \frac{(A540/A579\text{solution x}) - (A540/A579\text{solution z})}{(A540/A579\text{solution y}) - (A540/A579\text{solution z})} \times 100$$

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 as methaemoglobin (indicated by a relatively high absorbance in the region 580-600 nm).
- Lipaemic blood specimens may give turbid suspensions, which also give unreliable results.

Sensitivity

HbCO, approximately 10%

Treatment

- 100% oxygen

b) Food – born toxins

A microbial toxin is a compound produced by a microorganism that acts to cause disease. Food poisoning syndromes result after ingestion of a wide variety of foods contaminated with pathogenic microorganisms or microbial toxins. The pathogenic organisms are C*lostridium perferinges*, *Bacillus cereus, Escherichia coli, Closterdium botulinium* and *Vibrio cholerae*. Preformed toxins are from *Staphylococcus aureus, B.cereus and C.botulinum*. The illnesses produced usually are not associated with fever or blood, pus, or mucus in the stools because it doesn't have tissue involvement. Most of them produce GI symptoms. Except for botulism and cholera, the clinical course of most of these food-borne toxin related illnesses is self-limiting.

Laboratory analysis

General tests

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. perferingens* -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.

IV – DRUGS OF ABUSE

The term `drug abuse` connotes social disapproval. Any use of a drug for non-medical purposes, usually for altering consciousness but also for body building is known as abuse of drug. Psychological dependence (drug seeking behavior in which the individual uses the drug repetitively for personal satisfaction), physiologic dependence (withdrawal of the drug produces symptoms & signs), &tolerance (necessitating large doses of the drug to achieve the same response) are the main features of drugs of abuse**.** Some of the common drugs of abuse are discussed here.

a. Alcohols

Alcohol, primarily in the form of ethyl alcohol (ethanol), has occupied an important place in the history of human kind for at least 8000 years. Young children, chronic alcoholics or suicidal persons may ingest toxic quantities of one or several of the alcohols. Whether intentional or accidental, alcohol ingestions remain one of the more common, yet potentially devastating, poisonings commonly encountered in the emergency

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 µl 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.
2. Lightly stopper the tube and place in a boiling water-bath for 2 minutes.

Results

A change in colour from orange to green indicates the presence of volatile reducing agents such as ethanol; metaldehyde, methanol and paraldehyde.

Sensitivity

Ethanol, 0.5 g/l

Quantitative assay

Specimen

Whole blood, plasma, or serum (0.5 ml)

Reagents (see annex I)

1. Semicarbazide reagent.
2. Aqueous nicotinamide adenine dinucleotide (NAD) This solution is stable for 2-3 months at 4°C, but can be decomposed by vigorous agitation.
3. Alcohol dehydrogenase (ADH) suspension.
4 Aqueous perchloric acid

Standards

Solutions containing ethanol concentrations of 0.5, 1.0, 2.0 and 4.0 g/l prepared in heparinized whole blood to which 10 g/l sodium

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 concentration by analysis of the standard ethanol solutions and calculate the concentration of ethanol in the sample.

N. B -If the specimen contains an ethanol concentration of more than 4.0 g/l, the analysis should be repeated using a dilution (1:1 or 1:3) of the sample in blank plasma. Methanol does not interfere, but propan-2-ol and some higher alcohols will reduce NAD under the conditions used in this assay.

- In all cases where the analysis may be delayed, it is important to add 10 g/l sodium fluoride to the specimen to inhibit microbial biotransformation.

Sensitivity

Ethanol, 0.5 g/l

Treatment

- Symptomatic management
- Gastric lavage
- Hemodialysis
- In chronic toxicity thiamine can be given

b. Nicotine toxicity

Nicotine is one of the most widely abused chemical and now considered to be one of the most addicting substances. It is the principal pharmacologically active component of tobacco in which poisoning may occur in accidental ingestions of tobacco products (especially by children), use of nicotine-containing gums, and industrial exposure to tobacco products, contact with some pesticides and so on.

Nicotine has both stimulant and depressant action. Nicotine is readily absorbed through intact skin as well as through mucus membranes and the respiratory tract. It is metabolized by the liver and excreted by the kidney. Victims can complain of nausea, emesis, excessive salivation, and diarrhea at low doses. But at high dose it can cause respiratory paralysis, cardiovascular collapse, and convulsions.

There is no simple qualitative test for Nicotine, but this compound can be detected and identified by thin layer chromatography of a basic solvent extract of urine.

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 effect acting on opiate receptors located within the CNS resulting in analgesia & euphoria. Opioids are used to treat cough, diarrhea, dyspnea (congestive heart failure), and sometimes anxiety as well as pain. The most commonly abused drugs in this group are heroin, and morphine. Tolerance and dependence of opioids develop with chronic use. The classic triad for opioid poisoning is miosis, coma and respiratory depression.

Laboratory analysis

General tests

Arterial blood gas analysis (ABGs), CBC, electrolytes, BUN, creatinine and blood glucose measurements in victims with abnormal vital signs or mental status.

Toxic-specific tests

Quantitative opioid blood levels are not clinically useful. Qualitative analysis (screening) of the urine by thin-layer chromatography can detect some but not all opioids. Gas chromatography and enzyme-linked immunoassays or radioimmunoassay are more sensitive for detecting specific agents. Confirming the presence of a specific opioid is not necessary when the history and response to antidote (naloxone) are consistent with a generic diagnosis of opioid poisoning.

Specific laboratory tests

Qualitative test

PRINCIPLE

The Quick Screen One-Step Rapid Opiates Test technology (screen test for Morphine, Heroin, Codeine, &opium) incorporates a chromatographic absorbent device in which the drug or drug metabolites in the sample compete with an opiates derivative immobilized on a porous membrane for limited antibody sites. This is the preferred method for qualitative assay.

In the assay procedure, urine mixes with labeled antibody-dye conjugate and migrates through test device. When opiates levels

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 sample well”S” waiting 5 seconds between drops.
5. Observe migration or lateral flow of the sample across the entire test panel. Add additional sample drops if migration is not complete.
6. Positive results may be observed as soon as 5 minutes, depending on the concentration of opiates in the tested

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. *M*any 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 & mode of action, & there are far too many categories to cover in this short topic. So we like to give an overview on it.

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a. Animal toxins

Animal toxins comprise a diverse range of structures & modes of action. A simple & well known example is formic acid which is found in ants. Animal toxins are often mixtures of complex proteins. Most of us suffer from animal toxins at some time in our lives. However, in some countries death & illness due to animal poisons represents a significant proportion of cases.

Snake venom

Snake bite is one of the most common forms of poisoning by natural toxins world wide. The snake venom is a complex mixture of compounds. The enzymatic components of snake venom cause local and sometimes systemic effects, and the non-enzymatic components provide lethality. Absorption of snake venom is variable but most rapid through the blood vessels. Distribution depends on protein binding, membrane permeability and pH. The kidney excretes venom. Clinical presentations of snakebite may be obvious, but not always. It can cause anaphylactic reactions, nausea, vomiting, diarrhea, hemolytic anemia, hemorrhage, respiratory failure…

Laboratory tests

- Basic studies should be performed: CBC; platelet count; coagulation tests; electrolytes, creatinine and BUN levels.
- Urinalysis initially and with subsequent bedside checks using dip sticks can signal developing hematuria or myoglobinuria.
- Attempts to develop assays that identify the presence of venom at the puncture site or within the victim's serum have

met with little success. A radioimmunoassay was developed for this purpose, but it has never become a practical methodology and is useful only as a research tool. The enzyme-linked immunmosorbant assay bridges the gap because it can detect small amounts of antigen antibody complex.

Treatment

- Incision & suction (source of controversy because of tissue damage & it is better to use with a vacuum pump)
- Antivenoms (definitive)

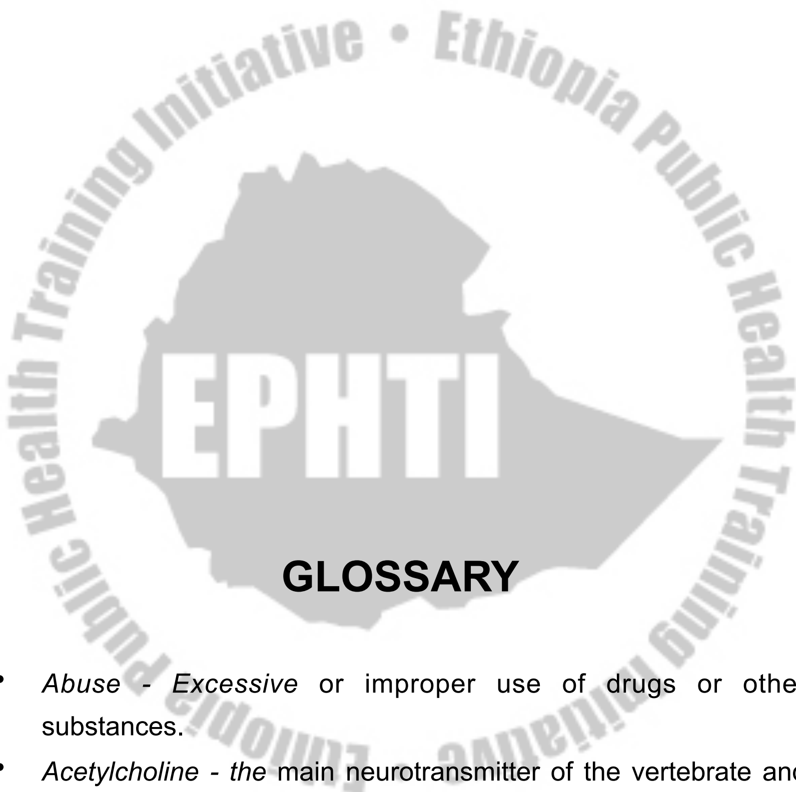
b) Plant toxins

Many species of plants contain toxic chemicals. There are many well known plant toxins ranging from the irritant formic acid found in nettles to more poisonous compounds such as atropine (atropa belladonna). The concentration of toxic chemicals is variable among the same species & different species. Major toxic effects are on the skin (e. g allergic dermatitis), GIT (e. g gastroenteritis), cardiovascular
(E .g arrhythmia)…

Exercise

1. What are industrial toxicants? What types of general laboratory diagnostic techniques are used?
2. What are medical toxicants? What kinds of laboratory techniques are used to identify them?
3. What are environmental toxicants? What kinds of laboratory techniques are used to identify them?
4. What are drugs of abuse? Discuss the laboratory techniques used to identify ethanol.

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.

Toxicology

- *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 (dysrrythmia)* -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 to an organic molecule (chelating agent) at two or more positions
- *Chelating agent* - A compound capable of forming a chelate with a metal ion.
- *Cholinesterase* - Enzyme that catalysis the breakdown of a choline ester to choline (*see also:* Acetylcholinesterase).
- *Chronic* - Long-term (cf. Acute).
- *Contaminant* - An impurity.

- *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.

- *Haemoglobin* -An iron-containing pigment found in erythrocytes, which binds oxygen for transport within the bloodstream.
- *Haemolysis*- Rupture of erythrocytes leading to the appearance of free haemoglobin in the plasma.
- *Herbicide*- A pesticide used to control or kill plants or plant seeds.
- *Hydrolysis* -Decomposition caused by or involving water.
- *Hyperglycaemia* -Abnormally high blood sugar (glucose) concentration.
- *Hyperventilation* -Increased rate and depth of respiration
- *Hypoglycaemia* -Abnormally low blood sugar (glucose) concentration.
- *Hypostatic*- caused by the combined effects of gravity & poor circulation of the blood.
- *Hypoxia* -Reduction of oxygen in an animal body below physiological requirements.
- *Metabolic acidosis -Acidosis* of metabolic origin.
- *Metabolite* -A substance produced by biotransformation.
- *Methaemoglobin* -Oxidized haemoglobin.
- *Miosis* -Contraction of the pupil of the eye.
- *Mixer, rotar- A* device for mixing solutions or suspensions by means of a gentle rotating motion. Used for solvent extraction or other procedures requiring mixing of relatively large quantities of material.
- *Opiate* - A pharmacologically active agent, such as morphine, derived from opium.
- *Osmolality* -The osmotic strength of a solution.

- *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 as plasma or serum. Reliable only for fluids with a viscosity similar to that of water.
- *Scene residue-* Material found at the scene of a poisoning incident.
- *Supernatant -* An upper layer of liquid.
- *Tolerance-* (i) The ability of an organism to experience exposure to potentially harmful amounts of a poison without showing evidence of toxicity. (ii) An adaptive state whereby the pharmacological effects of the same dose of a substance become diminished as a result of repeated exposure.
- *Toxic* -Able to cause injury to living organisms as a result of chemical interaction within the organism.
    - *Relative volume of distribution* -is volume of distribution divided by body weight.
    - *Respiratory acidosis- Acidosis* of respiratory origin.
    - *Vortex-mixer* -A device for mixing solutions or suspensions by means of a whirling motion which creates a cavity in the centre of the mixture. Used for solvent extraction and

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. Nitric acid- Sub-boiling redistilled ultra pure concentrated nitric acid is required to prepare the reagents.
b. Diluent- The diluent (and matrix modifier) is 10.0 mL/L Triton X-100 and 2.0-g/L ammonium dihydrogen phosphate in 0.2% nitric acid.
c. Calibrators- The calibrators are prepared from a stock reference solution containing lead at 1000 μg/mL, and then diluted further with diluent to achieve final concentrations of

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10, 20, 40, and 60 μg/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 µl of methanol and spot 20 µl on a column marked on the plate.

B. Spot 10 µl of the standard mixture on a second column.

C. Develop the chromatogram (10-cm run) using cyclohexane: acetone: chloroform (saturated tank) and allow to dry.

D. Spray the plate with 4-(*p*-nitrobenzyl) pyridine solution and heat, preferably in an oven, at 110°C for 30 minutes.

E. Allow to cool and spray with acetone: tetraethylenepentamine (9:1).

4. Reagent preparation in the qualitative test of aspirin

Trinder's reagent- Mix 40 g of mercuric chloride dissolved in 850 ml of purified water with 120 ml of aqueous hydrochloric acid (1mol/l) and 40 g of hydrated ferric nitrate, and dilute to 1 liter with purified water.

5. **Reagent preparation for quantitative assay of barbiturates**

A. Borate buffer, pH 8.4. Mix 22.4 g of disodium tetraborate with 76 ml of aqueous hydrochloric acid (1 mol/l) and dilute to 2 liters with purified water.

B. Aqueous hydrochloric acid (2 mol /l).

C. Concentrated sulfuric acid (relative density 1.83).
D. Concentrated ammonium hydroxide (relative density 0.88).
E. Sodium sulfate/charcoal mixture. Add 100 mg of activated charcoal to 100 g of anhydrous sodium sulfate, mix thoroughly and heat in an evaporating basin at 100°C for 8 hours. Allow to cool and store in a tightly stoppered bottle.

Standard solution preparation for qualitative assay of barbiturates

Solutions containing barbital at concentrations of 5, 10, 25 and 50 mg/l in blank human plasma, prepared by dilution from an aqueous solution of barbital sodium (1.12 g/l), equivalent to diethylbarbituric acid at a concentration of 1.00 g/l.

6. Reagent preparation for cholinesterase activity test

A. Dithiobisnitrobenzoate reagent. 5, 5'-Dithiobis (2-nitrobenzoic add) (0.2 g/l) in sodium dihydrogen orthophosphate buffer (0.1 mol/l, pH 7.4).
B. Aqueous acetylthiocholine iodide solution (5 g/l).
C. Aqueous pralidoxime chloride solution (200 g/l).
D. Plasma or serum from an unexposed individual (control plasma).

7. Reagent preparation for qualitative test of acetaminophen

A. Saturated *o*-cresol- Mix 10 mL of *o*-cresol with 1 L of deionized water. Allow to stand for 24 h before use. Solution will be stable for 1 year at room temperature.

B. Ammonium hydroxid, 4 mol /L - Dilute 284 mL of concentrated ammonium hydroxide to 1 L with deionized water. Solution will be stable for 1 year at room temperature.

C. Concentrated hydrochloric acid.

8. Reagents preparations for quantitative test of Acetaminophen

A. Acetaminophen stock reference solution, 1000 mg/L - Dissolve 100 mg of acetaminophen in 20 mL of methanol and then dilute to 100mL with deionized water. Solution is stable for at least 6 months when stored refrigerated.

B. Calibrator - Dilute the acetaminophen stock reference solution with deionized water to provide working calibrators of 20 and 100 μg/mL. Calibrators are stable for 2 months when stored refrigerated.

C. 3-Acetamidophenol stock internal standard, 1000 mg/L - Dissolve 100 mg of 3-acetamidophenol in 20 mL of methanol and dilute to 100 mL with deionized water. Solution is stable for 6 months when stored refrigerated.

D. 3-Acetaminophen working internal standard, 50 mg/ml. Dilute 5 mL of the stock internal standard to 100 mL with deionized water. Solution is stable for 2 months when stored refrigerated.

E. Phosphate buffer, 0.225 mol/L, pH 7.4 - Mix 80.4 mL of disodium hydrogen phosphate (Na_2HPO_4) solution (30.6 g/L) with 19.6 mL of potassium dihydrogen phosphate (KH_2PO_4) solution (31.9 g/L). Adjust to pH 7.4, if necessary, with the appropriate phosphate solution. Solution is stable for 2 months when stored at room temperature.

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-μm pore-size filter and degas by applying gentile vacuum

9. Reagents for quantitative test of ethanol.

A. Semicarbazide reagent.

Tetrasodium pyrophosphate dehydrated …….10 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. Alcohol dehydrogenase (ADH) suspension.

Ammonium sulfate……………………………….45.5 g
Tetra sodium pyrophosphate (dehydrated)……… 3 g
Distilled water…………………………………… 100 ml
Crystalline yeast ADH…………………………….2.5 g

Adjust pH to 7.3 with either aqueous hydrochloric acid or sodium hydroxide (both1 mol/l) this solution is stable for 2-3 months at 4°C.

D. Aqueous perchloric acid

Perchloric acid (700 ml/l) ………………………………..2.9 ml
Distilled water ……………………………………………100ml

ANNEX II

Apparatuses

1. Atomic Absorption spectrophotometer for quantitative analysis of Lead

- A good-quality graphite furnace atomic absorption spectrometer is required for analysis. The graphite furnace requires a L'vov platform to give optimal sensitivity and accuracy. Zeeman's background correction is useful to reduce the effect of background to a minimum.

2. Supplies for spectrophotometer for quantitative analysis of Lead

- Polypropylene pipette tips, specimen cups, and Teflon reagent storage bottles are required. Before use, they are leached in 10% nitric acid for 1 week and rinsed in triple-distilled water.

3. HPLC equipment for quantitative test of Acetaminophen

- A 250 x 4.5-mm –I.D. column containing octadecylsilane (C18) bonded-silica particles (5-μm diameter) is used for the analysis of acetaminophen. The HPLC system is equipped with a fixed-(254-nm) or variable-wavelength detector set at a sensitivity of 0.1 or 0.2 absorbance units full scale for

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acetaminophen concentrations of 0 to 200 or 200 to 500-μg/ 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 separating blood samples and solvent extracts
- Vortex-mixer or other form of mechanical or hand-driven shaker such as a rotary mixer
- Water-bath and (electrical) heating block
- Spirit lamp or butane gas burner
- Refrigerator (electrical or evaporative) for storing standards/samples
- PH meter
- Range of automatic and semi-automatic pipettes
- Low-power, polarizing microscope
- An adequate supply of laboratory glassware, including volumetric apparatus, and adequate cleaning facilities
- A supply of chemically pure water
- A supply of compressed air or nitrogen
- A supply of thin-layer chromatography plates or facilities for preparing such plates
- Facilities for developing and visualizing thin-layer chromatograms, including an ultraviolet lamp (254 nm and 366 nm) and a fume cupboard or hood
- Single-beam or dual-beam ultraviolet/visible spectrophotometer and associated cells

Toxicology

- Conway microdiffusion apparatus
- Porcelain spotting tile
- Modified Gutzeit apparatus

ANNEX III

Example of an analytical toxicology request form

<table>
<tr><td colspan="3" rowspan="2"></td><td>Date or admission :</td></tr>
<tr><td>Date/time of ingestion or exposure:</td></tr>
<tr><td colspan="3" rowspan="2">Doctor:
Telephone:
Hospital address for report:
Signed: Date:</td><td>Drugs prescribed or used in treatment</td></tr>
<tr><td rowspan="2">Drugs/poisons claimed or suspected (if possible with the suspected dose)</td></tr>
<tr><td colspan="3" rowspan="2">Victim:
Age/Date of birth: Sex:
Consultant: Ward:
Reference no:</td></tr>
<tr><td rowspan="2">Clinical details/ investigation required/</td></tr>
<tr><td>Sample type</td><td>Date</td><td>Time</td></tr>
</table>

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Toxicology

Blood (10ml heparinized)			priority:
Urine(50ml)			
Other(give details)			

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