FORENSIC SCIENCE STUDY MATERIAL

UNIT III

(CHEMICAL AND TOXICOLOGICAL ANALYSIS)

BY

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INTRODUCTION

Forensic toxicology deals with the investigation of drugs of abuse or toxic substances. This field involves toxicology and other disciplines such as biotechnology, pharmacology, analytical- and clinical chemistry to aid the legal investigation to find out the actual cause of death. Forensic toxicology incorporates a number of analytical techniques for detection of drugs from a variety of samples procured from the subjects. Forensic toxicology is a modern scientific field which involves the use of different analytical techniques like laser diode thermal desorption-tandem mass spectrometry (LDTD-MS-MS), Hyphenated liquid chromatographic techniques, Chromatography by silica-gel chromatobars, Ultra-high performance liquid chromatography-tandem mass spectrometry, DNA typing, and capillary electrophoresis. Forensic findings through these techniques may include the determination of pesticides, drugs, natural products, industrial chemicals, metals and pollutants. A toxicological analysis can be done to various kinds of samples procured from subjects under investigation. Blood, urine, nails, hair, bile, gastric contents, liver and brain tissue can all be useful specimens. There is a gradual increase in the demand of modern analytical techniques involved in forensic toxicology in solving disputes. The ever increasing popularity of the field of forensic toxicology in solving the criminal, suicidal and accidental cases has shown the importance of this particular field.

A. DRUGS OF ABUSE AND NARCOTIC DRUGS

DRUG According to "WHO"can be defined as "A Drug is any substance that is used or proposed to be used to modify or explore physiological structures or pathological states for the benefit of the recipient."

Eg: paracetamol, ciprofloxacin, sal-butamol, or it can be said that

A drug is a stuff which may have medicinal, intoxicating, performance augmenting or other effects, when taken or inserted into a human / animal body and which is not considered a food or a food supplement.

Drugs are defined differently by various drug control laws, government regulations, as medicine or on the basis of their usage.

The definition of drug in pharmacology can be stated as "a chemical substance used in the treatment, cure, prevention or diagnosis of disease or otherwise used to enhance physical or mental well-being." For chronic disorders, Drugs can be prescribed for a limited duration or on a regular basis.

Drugs of Hallucinogens and Opioids are the examples of *Recreational drugs,* which are chemical substances that *affect the CNS.* These type of drugs are abused for distinguished beneficial

effects on consciousness, perception, behavior and personality. Addiction and habituation is the beginning *drugs abuse.*

Drugs which are taken from outside the organism this usually distinguished from endogenous biochemical. For example, when hormone insulin is synthesized in the body; it is called a hormone when it is synthesized by the pancreas inside the body, but it is called a drug when it is introduced into the body from outside.

➢ CLASSIFICATION OF DRUGS

On the basis of the purpose of their use, different drugs can be classified into following two heads:

- Therapeutic Drugs
- Psychoactive Drugs

Although both of these categories; often overlap. Due to the specific usage and wide range, psychoactive drugs are treated as a distinct class.

❖ *Therapeutic Drugs*

A Therapeutic drug is a substance that has healing or preventive properties in relation to certain diseases, or is administered to enable a medical diagnosis. The drugs in common therapeutic use that may be classified chiefly into following four categories:

1) ***Analgesics and Antipyretics.*** An *analgesic* is a type of drug that relives pain. On the other part, an antipyretic is a type of drug that is used to reduce the temperature of the body. Aspirin and Paracetamol are the commonly used drugs in this category.

Aspirin (Acetylsalicylic Acid) is a white crystalline powder having an acidic taste and is used commonly in houses for pains, aches, etc. Even small doses of this drug may prove to be fatal due to *idiosyncrasy*. However, minimum fatal dose is about 5-10 grams.

Paracetamol (Acetaminophen) is a metabolite of phenacetin, and is widely in use these days in place aspirin. Ingestion of 20 tablets of 500 milligram each within three to five days is proved to be fatal.

2) Antihistaminic. These are the drugs which antagonize the action of histamine. These are commonly used in allergic disorders and other conditions like common cold. The common preparations include: Promethazine hydrochloride (Phenergan), Diphenhydramine (Benadryl), Chlorcyclizine (Histantin), Antazoline (Antistine), etc. Its fatal dose is about one gram.

3) Antidepressants. These are the drugs which are generally used in psychiatric disorders to treat the endogenous depression. These drugs have an initial sedative effect which is followed by an antidepressant effect within a week or more.

Commonly used antidepressant drugs are: Imipramine, Amitriptyline, etc.

4) Tranquilizers. These are the drugs that produce a general tranquility without the impairment of high- thinking facilities or the inducement of a sleep. To reduce tension and anxiety of mental patients Tranquilizers like reserpine and chlorpromazine are useful.

- ***Psychoactive Drugs***

A psychoactive drug, psycho-pharmaceutical or psychotropic drug is a chemical substance that acts principally upon the central nervous system where it affects brain function, resulting in modification in perception, mood, consciousness, cognition and behavior. These substances may be used recreationally, to purposefully change one's consciousness, or for ritual, spiritual or shamanic purposes. e.g., like anesthetics, analgesics or for the treatment of psychiatric disorders, these types of psychoactive drugs also have therapeutic activity.

Psychoactive drugs bring about marked changes in consciousness and mood that the user may find pleasant (e.g. euphoria) or advantageous (e.g. increased state of mind) and are thus, reinforcing. Despite health risks and neglecting consequences, many psychoactive substances are misused/ abused, that is, used excessively, with continuous use of some substances, psychological and physical dependence ("addiction") may develop.

Worldwide many governments posed restrictions on drug production and sales in an attempt to decrease drug abuse, due to misuse and physical dependency on drugs.

Classification of Psychoactive Drugs

Psychoactive substances are used by human beings for varied reason. Social sleep aids and social drinking are some of the examples. World’s largest consumption of psychoactive substance is caffeine, but unlike other drugs, it is unregulated and legal in almost all jurisdictions.

According to pharmacological effects in the body, psychoactive drugs are divided into different categories.

Generally used psychoactive drugs are:-

Narcotics, stimulants, Hallucinogens, Depressants and Anabolic steroids.

1) ***Narcotics.*** The term is originated from Greek word narkotikos that means 'a state of lethargy'. This group comprises of substances that act on the CNS and bring relief from discomfort and produce sleep. The origin of most narcotics is opium, a sticky milky juice obtained from the unripe pod of poppy (Papaver somniferium).

   Example: Opium, morphine, heroin, codeine, synthetic opiates, etc.

   Morphine is obtained from raw opium. It is normally administered by injection by hypodermic needle. It results in a euphoric state, with sleepy and relaxed appearance of the user. It is generally 3 to 5 times stronger than opium.

   Heroin (diacetylmorphine) is a white crystalline powder, which is derivative by adding two acetyl groups to the morphine, found in the opium. Heroin in impure form is known as Brown Sugar. It is 10-15 times more effective than morphine. It may be either injected or sniffed to cause similar effects as that of opium and heroin but with higher magnitude.

   Codeine is also a byproduct of morphine but is less effective as analgesic. It acts as a base in many pain relievers and cough remedies.

   Signs and symptoms of Narcotic abuse

   - ✓ Respiratory depression (shallow breathing), ¸
   - ✓ Small pupils, bloodshot eyes
   - ✓ Nausea, vomiting
   - ✓ Itching skin
   - ✓ flushed skin
   - ✓ Constipation
   - ✓ Poor judgment
   - ✓ Confusion

2) ***Stimulants*** ("uppers"). Stimulants consist of substances that, stimulates the mind, wakes one up and euphoria (a feeling of well-being), but do not affect perception. These drugs are also referred in the terminology of "speed".

   Examples: Amphetamines, methamphetamines, caffeine, nicotine, cocaine, etc.

   Amphetamine or its derivative *methamphetamine* may be injected direct into the blood stream through intravenous injection. The desire for a more intense experience is the chief motive behind this route of administration. First sensation of "flash" or "rush" initiates, followed by high feeling of pleasure, euphoria that produces hyperactivity, with a feeling of clarity of vision as well as hallucinations. After the effect wears off, the individual passes through a period of exhaustion and may sleep continuously for one to two days.

Another type of amphetamine is methamphetamine called “speed” because of its rapid stimulation of central nervous system.

Cocaine is also a potent stimulant that produces similar effects as that of amphetamines- namely, increased alertness, accompanied by suppression of hunger and fatigue. It is generally sniffed and is absorbed into the body by the mucous membrane of the nose.

One other form of cocaine which is quite popular is “crack”. It is manufactured by heating the mixture of cocaine, baking soda and water. It is also snorted and produces similar effects like cocaine.

3) ***Hallucinogens,*** including psychedelics, dissociatives and deliriants. This category comprises all those substances that produce distinct alterations in normal thought processes, perceptions and mood. There are a number of substances with varying chemical compositions that have hallucinogenic properties.

   Examples: LSD (lysergic acid diethylamide), PCP (phencyclidine), DMT (dimethyltryptamine), mescaline, psilocybin, etc.

   LSD is synthesized from lysergic acid, a substance derived from ergot, which is a type of fungus that attacks certain type of grains. This is a very potent drug, only 25 mg is sufficient to start clear visual hallucinations in the mind that may last for about 12 hours. This drug produces noticeable changes in mood, leading to laughing and crying at the slightest provocation. Feeling of anxiety and tension always accompanied LSD use.

   On the other hand, PCP is synthesized by quite a simple chemical process, thus is much more easily available. PCP is frequently mixed with other drugs like amphetamine or LSD, and is sold as a Powder (“Angel Dust”), capsules or tablets, or as a liquid. The drug is smoked, ingested or sniffed.

   Marijuana is an example of a psychoactive drug that combines properties of each of these groups.

   Cannabis plant (charas, bhaang, ganja & hashish)

4) ***Depressants*** ("downers"), including sedatives, hypnotics, and narcotics. This category consist of all of the sleep inducing, calmative, anxiety-reducing, anesthetizing substances, which induces perceptual changes like dream sequences, and also often bring to the mind the feelings of euphoria.

   Examples: Alcoholic beverages (ethanol), barbiturates, benzodiazepines, etc.

Like alcohol, barbiturates act on central nervous system to suppress its vital functions so relax, create a feeling of well being and produces sleep. Barbiturates are usually administered by mouth. The average sedative dose is about 10-70 milligrams. There are about 25 barbiturate derivatives.

Some barbiturates are absorbed more slowly than others and are generally termed as "Long acting barbiturates" like Phenobarbital. On the other part, some barbiturates are absorbed rather quickly and are termed as "Short acting barbiturates" like Pentobarbital, Sec barbital, etc. Apparently, abusers prefer the faster acting ones.

B. TOXICOLOGICAL EXAMINATION OF POISIONS AND ALCHOHAL

1. TOXICOLOGICAL EXAMINATION OF POISION

Death by poison can happen in a variety of ways, for example through recreational exposure by inhaling solvents such as butane lighter fluid or fuels, ingesting plant-derived substances like Angel's Trumpet, accidental exposure to a substance used in the workplace or even accidently produced in the home (like carbon monoxide), or suicidal ingestion of a poison such as strychnine, pesticides, cyanide, etc. These all require specialized tests and the laboratory is alerted to their possible usage or involvement in the death when requests for toxicology testing are submitted.

Specimens sent for toxicology testing are usually collected by the forensic pathologist (who may also be an appointed "medical examiner" or "coroner" in some jurisdictions) or mortuary technician during an autopsy. Specimens must be properly identified, labelled and sealed as soon as practicable after collection. All specimens pertaining to a case must be collected and bagged separately in tamper-proof containers. Unique numbered seals are used to track all evidence for each case. Like any other evidence, the chain of custody must be preserved at all times, from the mortuary through the laboratory testing, reporting and storage, for court purposes. If the continuity of evidence is compromised, it can result in the case being dismissed in court.

- **Specimen collection**

Table 1 (below) provides a snapshot of the kinds of samples commonly requested when investigating different manners of death, although there may be unique case needs that have to be addressed for some investigations. For example, exposure to volatile substances

requires a sample of the fluid in the lung. Skeletal remains can be useful to determine prior exposure to drugs and other substances. In these cases hair can also be sampled.

Table 1. Recommended specimens collected in post-mortem cases.

Type of death case	*Recommended specimens*
Suicides, motor vehicle crashes, and industrial accidents	Blood, urine, vitreous humour, liver
Homicides and/or suspicious	Blood, urine, vitreous humour, gastric contents, bile, liver, hair
Drug-related	Blood, urine, vitreous humour, gastric contents, bile, liver, hair
Volatile substance abuse	Blood, urine, vitreous humour, lung fluid or tied-off lung, liver
Heavy metal poisoning and exposure to other poisons	Blood, urine, vitreous humour, liver, hair, kidney

Blood, Urine, Liver - Blood is often the specimen of choice for detecting, quantifying and interpreting drugs and other toxicant concentrations. Concentrations of drugs and other toxicants in blood may be useful for establishing recent drug ingestion and to determine the effect of a drug on the deceased at the time of death, or at the time the blood was taken. This can complicate the investigation when someone has been taking prescription medications for some time. For cases involving hospital treatment before death, blood samples taken soon after admission and immediately before death, should also be investigated particularly when poisoning is suspected before admission into hospital. Any treatment given can change the results of toxicology tests or be helpful in the investigation.

Post-mortem blood presents problems due to often variable condition and changes to concentrations from one place to another in the body after death. The degree of decomposition can also interfere with testing as these specimens can be difficult to analyse.

Urine is the most common sample used for drug testing in the workplace, but it is not always available for post-mortem testing. Urine testing results do not directly correlate to drug effects at the time of sample collection because of the time it takes the body to eliminate these drugs or their metabolites (the body's breakdown products) in the urine. Its usefulness

lies in the fact that the presence of a substance in the urine is a sign that the substance had been in the blood at an earlier time (usually within a few days) and had been somewhat processed (detecting these metabolites gives proof that the drug had been ingested). When urine is available, tests are also conducted for presence of drugs of abuse. Depending on the case and the results of initial testing other tissues may need to be analyzed, possibly including stomach contents, liver, etc., (see Table 1).

The liver is a primary solid tissue for use in post-mortem toxicology because it is where the body metabolizes most drugs and toxicants. Many drugs become concentrated in the liver and can be found even when there are no levels in the blood. In this latter situation, interpretation of findings is complex.

Vitreous Humour - is the clear, gel-like substance that fills the eye. It can be a useful fluid to screen for a range of drugs. Vitreous humour is commonly analyzed for blood alcohol concentrations. This is of particular interest in motor vehicle trauma, workplace accidents, suicides and homicides. Vitreous humour alcohol concentrations are a little higher than blood (about 20% on average), assuming there has been no degradation. Interpretation of other toxicological findings in vitreous humour is somewhat more complex.

- **Other samples less commonly used for post-mortem toxicology**

Stomach Contents - Because drugs and poisons can often be ingested, stomach contents can provide important investigative clues. In a case of potential overdose or acute poisoning, high concentrations of drugs or toxins may be detected, depending on how much time elapsed between ingestion and death. In many cases of acute poisoning, undissolved capsules or tablets may be discovered, allowing relatively simple drug or poison identification. The total amount of a drug or poison present in the stomach is more important than its concentration because it has not been processed by the body yet.

Bone and Bone Marrow - Bone, in particular bone marrow, can be used for testing when necessary, but the availability and condition of bones in skeletal remains may limit their usefulness. There are no data to suggest that bones from one part of the body are better than others for toxicology tests. However, it is always easier to extract samples from larger bones. Interpretation of these findings is often difficult when assisting in a death investigation, because the time that these toxins were deposited in the bones cannot be determined with reasonable certainty.

Hair and Nails - Hair specimens, usually taken from the back of the head, can be used to test for exposure to heavy metals and drugs over a period of weeks to months. Hair is predominantly used to test for drugs such as amphetamines, cocaine, marijuana (THC) and heroin, and more recently tests have been created to determine if the deceased was drinking heavily in the last few months before death. Drug analysis can also be done on finger- and

toenails in order to provide an even longer potential window of exposure than hair. However, relatively little is known about how the nails process toxins, so interpretation of results is more difficult. Hair is subject to external contamination issues that can mitigate its value, so special sample preparations in the lab may be needed for a given case.

2. TOXICOLOGICAL EXAMINATION OF ALCHOHAL

The term 'alcohol' is derived from an Arabic word "Al kohl", which means "something subtle". Alcohol causes a slowing of nerve conduction, which results in slower reaction times, difficulty in processing and assimilating information. The aliphatic alcohol forms a homologous series beginning with methanol, ethanol, n-propanol, isopropanol etc. The first three are readily soluble in water in all proportions but as the carbon chain length increases, water solubility decreases and Octanol is almost insoluble in water. Alcohol beverages are principally a mixture of water and ethyl alcohol with small amounts of other substances, which impart the characteristic odour and flavour to the beverage. These substances are referred as Congeners because they are simultaneously produced during the fermentation process. Congeners consist of organic acids and esters. Alcohol is one of the most useful alcohols for many purposes. Being a central nervous system depressant, it causes an irregularly descending type of depression. Higher centres are depressed first followed by the midbrain and thalamus, spinal cord and finally the medulla. The recovery occurs in a reverse order.

- **Forensic Issues**

Alcohol is undoubtedly still the largest contributor to impaired driving, but the additional impact of drug impairment on driving has also become a focus in recent years. Forensic toxicologists play a significant role in many aspects of drunken driving, particularly in measuring the pharmacological relationship between drug or alcohol use and impairment. They have a crucial role in analysing samples from motorists suspected of being impaired, interpreting results and presenting material evidence in the court. Ethanol is the most commonly encountered alcohol in terms of drunken driving cases. Consumption of ethanol is legal in many jurisdictions where it is normally considered as socially acceptable if consumed in moderation.

Other alcohols are occasionally encountered in terms of drunken driving cases. Methanol, is a highly toxic substance and is not generally consumed knowingly but may be consumed by accident, particularly by persons desperate to consume alcohol who are unaware of the toxicity. Methanol is also of major concern as it is frequently been used for adulterating liquors as a cheap substitute of ethanol in the illicit liquor manufacture syndicates, which generally results in fatal cases. Other alcohols such as isopropanol and ethylene glycol can cause impairment but, as for methanol, consumption of these substances is uncommon.

Products of alcohol	Alcohol by volume
Rum	50 – 60 %
Whisky, Gin, Brandy	40 – 45 %
Port, Sherry	16 – 20 %
Wine	10 – 15 %
Beers	4 – 8 %
Arrack	40 – 50 %

SOME NOTABLE ALCOHOLS OF FORENSIC IMPORTANCE

1. Ethanol

Ethanol (Ethyl Alcohol) or Grain Alcohol is clear, colourless liquid with typical fruity odour and having burning taste. It is produced mostly by synthetic production from Ethylene. This is mainly done by direct hydration process. Ethanol is soluble in water as well as lipid. The hydroxyl and ethyl groups confer both hydrophilic and lipophilic properties. Thus Ethanol is an "amphophyle". Its specific gravity is 0.79, which is 1 ml of alcohol weighs 0.79 gm. The ethanol content of various alcoholic beverages is expressed by volume percentage or by proof, the latter being twice the percentage of alcohol by volume. Ethanol is toxic by oral, inhalation, sub-cutaneous, intravenous, intra-arterial, intraperitoneal, and dermal routes. Vaporized ethanol can be rapidly absorbed by inhalation leading to intoxication. Ethanol is a CNS depressant but produces some apparently stimulating effects initially because of depression of inhibitory control mechanisms in the brain. Several antihistaminic, decongestant, multivitamin, and cough syrups contain varying percentage of alcohol from 2 to 25 %. Ethanol has been popular in the past as an antiseptic. Surgical spirit used even today is mostly ethanol with a small quantity of methanol (90 to 95% and 5 to 10 % respectively), along with traces of Castor Oil and Methyl Salicylate.

2. Methanol

Methyl alcohol is prepared by destructive distillation of wood or molasses. It burns with pale blue non-luminous flame and its vapour produces explosive mixture in presence of oxygen or air. Methanol, also known as Methyl Alcohol or Wood Alcohol, is a chemical compound with chemical formula CH3OH. It is the simplest alcohol, and is a light, volatile, colourless, tasteless, flammable, poisonous liquid with a very faint odour. It is used as an antifreeze, solvent, fuel and as a denaturant for ethyl alcohol. Because of its poisonous properties, methanol is also used as a denaturant for ethanol. Methanol is rapidly absorbed from the gastrointestinal tract, through

lungs and skin. Methanol itself is not toxic but two metabolites formed, formaldehyde and formic acid are highly toxic. These compounds are responsible for causing profound metabolic acidosis and visual defect and blindness.

3. Isopropanol

Isopropanol or Isopropyl alcohol or 2-propanol is also commonly known as 'Blue heaven' as isopropanol is often highlighted blue to differentiate it from other colourless liquids, which has led to the designation "blue heaven" by the addicts. However, it is a colourless, volatile liquid with a faint odour of acetone and a slightly bitter taste. It is generally used, in massage as Rubbing Alcohol (70%), as a Disinfectant, as Antifreeze, as Paint remover, as cleaning solution, in Toiletries like hair tonics, as after-shave lotion, and as an Industrial solvent. Isopropanol is two to three times more potent than ethanol as a CNS depressant and the usual Fatal Dose of Isopropanol is about 250 to 300 ml. Isopropanol can be absorbed through all routes. In the body it is rapidly metabolised by alcohol dehydrogenase. Approximately 80% is converted to acetone and the residue is excreted unchanged in the urine. Acetone is excreted in the urine and breath, and also metabolised to acetate, formate, and carbon dioxide. Isopropanol may be generated spontaneously in a dead body, presumably due to bacterial or other putrefaction processes. This fact must be borne in mind when subjecting viscera to chemical analysis.

4. Ethylene Glycol

Ethylene is also known as 1, 2-Ethanediol or Glycol alcohol. It is a colourless, thick, odourless, non-volatile liquid, with a bittersweet taste. Ethylene glycol is not absorbed through skin, and because of its low vapour pressure does not produce toxicity upon inhalation. It is however rapidly absorbed through the Gastro Intestinal tract and is metabolised (more than 80%) to Glycoaldehyde, Glycolic Acid, and Oxalic Acid which inhibit diverse metabolic pathways in the body, including oxidative phosphorylation. Other metabolites include Glyoxylic Acid, Glyoxal, Formic Acid, Glycine, Oxalomalate, Malate, Benzoic Acid, and Hippuric Acid. The usual fatal dose of Ethylene Glycol is about 70 to 100 ml.

FORENSIC EXAMINATION OF ALCOHOL

1. Ethanol (Ethyl Alcohol)

For the detection of ethanol, following tests are to be carried out in the exhibits.

- **Iodoform Test:**

Appropriate amount of the sample is taken and about 1 ml of 5% Sodium Hydroxide solution is added to it and subsequently Iodine solution is added drop-wise with shaking until the liquid becomes persistent dark brown in colour. The whole is left for few minutes. If the Iodine colour disappears, more drops of Iodine solution are added drop wise until persistent brown colour of iodine reappears. Few drops of dilute Sodium Hydroxide solution are added to remove extra Iodine. Equal volume of water is added and left for about ten minutes. Observation of Yellow crystalline precipitate indicates the positive test for the presence of Ethanol.

- **Dichromate Test:**

Adequate amount of sample is taken and about 0.2 ml of 2% Potassium Dichromate solution is added followed by about 1 ml of concentration Sulphuric Acid. The yellow colour of the dichromate changes to green or blue indicates the presence of Ethanol.

- **Sulphomolybdic Test:**

Sulphomolybdic Acid is actually 1 gm of molybdic acid in 25 ml. of concentrated sulphuric acid. Now for the detection, 2 ml. of the hot reagent is added to 2 ml. of the distillate. A deep blue ring appears at once at the junction of two liquids. On shaking, the whole mixture becomes deep blue. It indicates the presence of Ethyl Alcohol. This test is highly sensitive and is negative with acetone, acetaldehyde and even dilute solution of methyl alcohol. Strong solution of methyl alcohol gives only a light blue colour after several minutes.

2. Methanol (Methyl Alcohol)

For the detection of ethanol, following tests are to be carried out in the exhibits.

- **Chromotropic Acid Test:**

Appropriate amount of sample is taken in a test tube and about 2 ml of Potassium Permanganate solution is added to it and shaken well. After that few crystals of Sodium Bisulphate is added with shaking till the disappearance of colour of the solution. About 1 ml of Chromotropic Acid, i.e., 5% of aqueous solution of Sodium salt of Chromotropic Acid and subsequently concentrate Sulphuric Acid is added slowly with inner sidewall of the test tube to the extent of 15 ml. Appearance of violet colour indicates the presence of Methanol.

- **Schiff's Reagent Test:**

About 4.5 ml of sample is taken in a test tube and 0.5 ml of Ethanol is taken. 2 ml of 3% Potassium Permanganate solution and 2ml of Phosphoric Acid is added to it and left for 10 minutes. After that 1 ml of 10% Oxalic Acid is added followed by 1ml of concentrated Sulphuric Acid. The contents are left to cool at room temperature. 5 ml of Schiff's reagent is added further and kept for half an hour. Appearance of purple colour indicates positive test for the presence of Methanol.

3. Isopropanol (Iso propyl Alcohol)

The acid-distillation of the exhibit is used to separate isopropyl alcohol from tissue and other biological material. The distillate is subjected to the following test:

2 ml. of distillate is taken in each of two test tubes and 3 drops of Potassium Permanganate in Phosphoric Acid (by dissolving 3 gms. of $KMnO_4$ and 15 ml. of syrupy Phosphoric Acid in 85 ml. of water) is added in one of the test tubes. This is allowed to stand for 5 minutes. The colour, if any left after oxidation is decolourised with a pinch of Sodium Bisulphite. After that 1 ml of 10% Sodium Hydroxide and 1 ml. of 5% Furfural are added to both the test tubes. The contents of each of the test tubes are filtered into test tubes containing 2 ml. of conc. Hydrochloric Acid. A pink ring is formed at the junction indicates the presence of Isopropanol and the pink colour in the other test tube indicates presence of Acetone.

3 TOXICOLOGICAL ANALYSIS OF VISCERA

A toxicological examination of viscera is a chemical and toxicological analysis of internal organs, body fluids, and secretions to determine if poisoning or intoxication was the cause of death. This analysis is performed in a Forensic Science Laboratory by a pathologist during an autopsy.

A toxicological examination of viscera is a chemical and toxicological analysis of internal organs, body fluids, and secretions that's performed in a forensic science lab to determine if a person died from poisoning or intoxication. This analysis is a routine part of a complete autopsy.

The purpose of the analysis is to detect the presence of substances like drugs or alcohol that may have contributed to the death. The analysis is important because it can help confirm or refute statements made by witnesses during a police investigation.

Here are some things to consider when performing a toxicological examination of viscera:

- **Sample collection**: It's important to collect representative samples.
- **Preservation**: The samples must be preserved properly to avoid artifacts that could affect the results.
- **Analysis**: The analysis must be performed with a high degree of precision.
- **Interpretation**: The results must be interpreted carefully, taking into account the possibility of artifacts.
- **Substances to consider**: Some substances, like proteinous poisons, are difficult to extract and detect. For substances that are normally present in the body, a range of values must be considered before interpreting the results.

The medical officer is responsible for determining the cause of death and the time since death. The toxicologist can only indicate that a potentially toxic substance was taken and estimate the quantity.

	Details
Purpose	To rule out poisoning or intoxication as the cause of death
Samples	Viscera and body fluids, such as blood, liver, spleen, and kidney
Analysis	Tests for drugs, alcohol, pesticides, phosphine, and other toxic substances
Preservation	Samples must be preserved in an appropriate preservative in sealed containers
Histopathology	Microscopic examination of tissues can help substantiate a suspicion of long standing abuse

Heavy metals	Viscera samples need to be digested before analyzing for heavy metals

The toxicologist can state that a potentially toxic substance was taken and estimate the quantity taken. If the quantity is in the range of a toxic or lethal dose, and other findings fit, then it can be said that poisoning is the cause of death.

4. TOXICOLOGICAL ANALYSIS OF PETROLEUM PRODUCTS

➢ WHAT ARE TOTAL PETROLEUM HYDROCARBONS?

Total Petroleum Hydrocarbons (TPH) is a term used to describe a broad family of several hundred chemical compounds that originally come from crude oil. In this sense, TPH is really a mixture of chemicals. They are called hydrocarbons because almost all of them are made entirely from hydrogen and carbon. Crude oils can vary in how much of each chemical they contain, and so can the petroleum products that are made from crude oils. Most products that contain TPH will bum. Some are clear or light-colored liquids that evaporate easily, and others are thick, dark liquids or semi-solids that do not evaporate. Many of these products have characteristic gasoline, kerosene, or oily odors. Because modern society uses so many petroleum-based products (for example, gasoline, kerosene, fuel oil, mineral oil, and asphalt), contamination of the environment by them is potentially widespread. Contamination caused by petroleum products will contain a variety of these hydrocarbons. Because there are so many, it is not usually practical to measure each one individually. However, it is useful to measure the total amount of all hydrocarbons found together in a particular sample of soil, water, or air.

The amount of TPH found in a sample is useful as a general indicator of petroleum contamination at that site. However, this TPH measurement or number tells us little about how the particular petroleum hydrocarbons in the sample may affect people, animals, and plants. By dividing TPH into groups of petroleum hydrocarbons that act alike in the soil or water, scientists can better know what happens to them. These groups are called petroleum hydrocarbon fractions. Each fraction contains many individual compounds. Much of the information in this profile talks about TPH fractions

➢ MEDICAL TEST TO DETERMINE EXPOSURE TO TPH

There is no medical test that shows if you have been exposed to TPH. However, there are methods to determine if you have been exposed to some TPH compounds, fractions, or petroleum products. For example, a breakdown product of *n*-hexane can be measured in the urine. Benzene can be measured in exhaled air and a metabolite of benzene, phenol, can be measured in urine to show exposure to gasoline or to the TPH fraction containing benzene. Exposure to kerosene or gasoline can be determined by its smell on the breath or clothing. Methods also exist to determine if you have been exposed to other TPH compounds. For example, ethylbenzene can-be measured in the blood, urine, breath, and some body tissues of exposed people. However, many of these tests may not be available in your doctor's office.

If you have TPH compounds in your body, they could be from exposure to many different products, and tests cannot determine exactly what you were exposed to. Tests are useful if you suspect that you were exposed to a particular product or waste that contains TPH.

There are a number of methods for detecting petroleum products in the human body, including:

- **Smell**: A characteristic odor of petroleum on the person's breath or clothing can indicate hydrocarbon poisoning.

- **Urine tests**: A breakdown product of n-hexane can be measured in urine, and a metabolite of benzene, phenol, can also be measured in urine.

- **Blood tests**: Ethylbenzene can be measured in the blood of exposed people.

- **Exhaled air tests**: Benzene can be measured in exhaled air.

- **Gas chromatography (GC)**: GC is the primary method for detecting kerosene in biological materials like blood. GC can be combined with mass spectroscopy (MS) for peak identification.

- **Chest x-ray**: A chest x-ray can be used to diagnose pneumonia and chemical pneumonitis.

- **Blood gas analysis**: Blood gas analysis can be used to diagnose pneumonia and chemical pneumonitis.

- **Magnetic resonance imaging (MRI)**: An MRI can be used if doctors suspect brain damage.

Petroleum products can have a number of effects on the body, including:

- Pulmonary effects
- CNS effects, such as slurred speech, disorientation, headache, dizziness, and nausea
- Arrhythmias
- GI tract irritation and breakdown of the epithelium, leading to nausea, vomiting, abdominal pain, and hematemesis
- Metabolic acidosis

5. TOXICOLOGICAL EXAMINATION OF FOOD ADULTERARION

Food adulteration

Adulteration of food commonly defined as "the addition or deletion of any substance to or from food, so that the natural composition and quality of food substance is affected. Adulteration is either intentional by either removing substances to food or altering the existing natural properties of food knowingly. Unintentional adulteration is usually attributed to ignorance, carelessness or lack of facilities for maintaining food quality.

Food Adulterants are the substances which are added to food items for economic and technical benefits but they reduce the value of nutrients in food and also causes the food contamination leads to unfit for consumption. Food adulterants could be available in dairy products, cereal products, meat & eggs, canned & bottled vegetables, fruits & fruit products fats & oils, beverages etc.

Food is declared adulterated if:

- A substance is added which depreciates or injuriously affects it.
- Cheaper or inferior substances are substituted wholly or in part.
- Any valuable or necessary constituent has been wholly or in part abstracted.

- It is an imitation.
- It is colored or otherwise treated, to improve its appearance or if it contains any added substance injurious to health.
- For whatever reasons its quality is below the Standard

Very often food is adulterated by merchants and traders who are dishonest and want to make a quick profit. But shortages and increasing prices, consumer demands for variety in foods, a lack of awareness, negligence, indifference and lethargy among consumers and inadequate enforcement of food laws and food safety measures also lead to food adulteration.

Types of Adulterants

Majority of adulterants used by the shopkeepers are cheap substitutes easily available. Classification of adulterants based on purpose

I. **Intentional Adulterants:** Sand, marble chips, stones, mud, other filth, talc, chalk powder, water, mineral oil and harmful colour.

II. **Incidental adulterants:** Pesticide residues, droppings of rodents, larvae in foods.

III. **Metallic contaminants:** Arsenic from pesticides, lead from water, effluent from chemical industries, tin from cans.

Secondary classification of adulterants based on effects

Poisonous or Deleterious Substances: Generally, if a food contains a poisonous or deleterious substance that may render it injurious to health, it is adulterated. If a food contains a poisonous substance in excess of a tolerance, regulatory limit, or action level, mixing it with "clean" food to reduce the level of contamination is not allowed. The deliberate mixing of adulterated food with good food renders the finished product adulterated.

Filth and Foreign Matter: Filth and irrelevant material include any objectionable substances in foods, such as foreign matter (for example, glass, metal, plastic, wood, stones, sand, cigarette butts, etc), undesirable parts of the raw plant material (such as stems, pits in pitted olives, pieces of shell in canned oysters), and filth (namely, mold, rot, insect and rodent parts, excreta, decomposition).

Economic Adulteration: A food is adulterated if it omits a valuable constituent or substitutes another substance, in whole or in part, for a valuable constituent (for instance, olive oil is diluted with tea tree oil); conceals damage or inferiority in any manner (such as fresh fruit with food

coloring on its surface to conceal defects); or any substance has been added to it or packed with it to increase its bulk or weight, reduce its quality or strength, or make it appear bigger or of greater value than it is (for example, scallops to which water has been added to make them heavier).

Microbiological Contamination and Adulteration: The fact that a food is contaminated with pathogens (harmful microorganisms such as bacteria, viruses, or protozoa) render it adulterated. Generally, for ready-to-eat foods, the presence of pathogens will render the food adulterated. For example, the presence of *Salmonella* on fresh fruits or vegetables or in ready-to- eat meat or poultry products will render those products adulterated.

Forensic Analysis of some common food products

- **Analysis of Sugar**

For the detection of physical impurities, small amount of sugar is taken in a test tube and agitated with little water. Pure sugar is dissolved in water but insoluble impurities do not dissolve. For the detection of adulteration by using chalk powder or washing soda in sugar, small amount of sugar is taken in a test tube and few drops of dilute Hydrochloric Acid are added. A brisk effervescence of Carbon Dioxide confirms the presence of chalk powder or washing soda in the given sample of sugar.

- **Analysis of Milk Products**

1. Ghee

Usually, vanaspati oil is added with Ghee in order to adulterate a high value product to gain more by adding comparatively less valued product. For the detection of vanaspati in ghee, small amount of suspected sample is taken in a test tube and heated gently. Small amount of sugar and Hydrochloric Acid is added to it. The test tube is then shaken well for atleast five minutes. The presence of pink colour confirms the presence of vanaspati oil in ghee

2. Paneer or Khoya

Small portion of the suspected product is taken in test tube, added water and boil. It is then cooled at room temperature. Few drops of Iodine solution is added to it. Blue colour indicates the presence of starch.

- ***Analysis of Edible Oil***

To detect adulteration of paraffin wax and hydrocarbons in vegetable ghee, small amount of unsaponifiable matter of oil is heated with Acetic Anhydride in a test tube. Small droplets of oil observed on the surface of unused Acetic Anhydride indicate the adulteration of oil with paraffin wax or hydrocarbon.

For the detection of Argemone oil in mustard oil, about 5ml of suspected oil is taken in a test tube. Few drops of concentrated Nitric Acid are added into it and contents are shaken well. Presence of orange colour indicates the presence of argemone oil in the suspected sample.

- ***Analysis of Spices and Condiments***

1. *Analysis of Chilli Powder*

For the detection of Red Lead salts mixed in the Red chilli powder, dilute Nitric Acid is added to the suspected sample and filtered. Two drops of Potassium Iodide is added into it. The presence of Yellow colored precipitate indicates the presence of red lead salts into the sample. Now, for the detection of red brick dust, the sample is dissolved in water in a beaker. Settling of some part in the bottom of the beaker and floating of some part over water indicates the presence of brick powder in the sample.

2. *Analysis of Turmeric Powder*

Chalk powder is often mixed with turmeric powder and for its detection, small amount of suspected sample is taken in a test tube and dilute Hydrochloric Acid is added to it. No effervescence indicates the absence of chalk powder in the sample.