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#### Chapter

### Forensic Chemistry and Toxicology

Amarnath Mishra

# Abstract Chopen

The current chapter deals with forensic chemistry & toxicology which is completely based on the introduction and classification of poisons and their impacts on the body and the factors affecting them and detection and examination of poisons. The purpose of this chapter is to discuss their mode of action and function once they reached in the human body. The impacts of poisons are severe and even cause death if not treated properly.

Keywords: poison, mode of action, alcohol, forensic analysis

#### 1. Introduction

Four hundred years back, Paracelsus stated that, "All substances are poisons; there is none which is not a poison." If the right dose is taken, it could become a remedy, otherwise poisonous [1, 2]. The therapeutic index or ratio, i.e., LD50/ ED50, tells whether the chemical is safe or not.

Poisons are generally found in cases of homicides, suicides, or accidents. They have a significant role to play as the silent weapon to destroy life mysteriously and secretively.

#### 2. Action of poisons

Every poison has almost similar action on the victim's body. In many cases, they either stop the transfer of  $O_2$  to the tissues or create an obstacle in the respiratory system by inhibition of enzymes which are associated with the process. In this, the myoneural junction and the ganglions and synapses are the sites of action. In some cases of insecticidal poisoning, hyperexcitement of voluntary and involuntary muscles can cause death. There are four categories of action of poisons—(i) local action, (ii) remote action, (iii) local and remote actions, and (iv) general action.

**Local action:** Local action means direct action on the affected site of the body. Examples include irritation and inflammation in strong mineral acids and alkalis, congestion and inflammation by irritants, the effect on motor and sensory nerves, etc.

**Remote action:** Remote action affects the person due to absorption of that poison into the system of that person. For example, alcohol is absorbed in the system and then it affects the person.

**Local and remote actions:** Some poisons can affect both local and remote organs. Thus, they not only affect the area with contact to the poison but also cause toxic effect after absorption into the system, for example, oxalic acid.

**General action:** General action means the absorbed poison affects more than one system of the body, for example, mercury, arsenic, etc.

#### 3. Factors modifying the action of poisons

Toxicity of a poison depends upon its inherent properties such as physiochemical as well as pharmacological properties.

The action of poisons mainly depends upon the following factors discussed below:

1. Forms of poison: There are three forms of poison:

• **Physical form:** Gaseous/volatile/vaporous forms of poisons act faster than liquid poisons as they are quickly absorbed. Similarly, liquid poisons act faster than solid poisons.

Gaseous or volatile > liquid > solid.

For solid poisons, powdered poisons act quickly than the lumps. For example, there are certain seeds that escape the gastrointestinal tract as they are solid, but when crushed, they can be fatal.

For solids: powdered > lumps

• **Chemical form:** Few substances like mercury or arsenic are not poisonous as they are insoluble and cannot be absorbed when they are in combination with other substances like mercuric chloride, arsenic oxide, etc.

In other cases, the action is vice versa. For example, there are some substances that become inert in combination with silver nitrate and hydrochloric acid and are deadly and poisonous when present in pure forms.

- **Mechanical combination:** The effect of poisons is significantly altered when they are combined with inert substances.
- 2. Quantity: Large doses of toxin cause much lethal effect. But this statement is not always true. For example, sometimes when a toxin is taken in very large amount, the body produces a mechanism against it such as vomiting, and thus the intensity of the toxin is reduced.
- 3. **Concentration:** The absorption speed of poison is dependent on concentration; thus poison of higher concentration is fatal. However, there are still some exceptions. For example, a dilute oxalic acid is less corrosive, but the absorption rate is high and so it is more dangerous.
- 4. **Methods of administration:** It has a unique role in the process of absorption. It is fastest through inhalation and then through injection as compared to the oral mode.
- 5. **Condition of the body:** Different persons react differently when exposed to a poison. It is because the condition of our body is also responsible for the increase or decrease of the effect of a poison on the body:
  - **Age**: Children and older people are more affected than an adult by the same quantity of toxin.

- **Sleep**: The body functions are slower during sleep; thus toxin circulation in the body is also slower.
- **Health**: Healthy persons can tolerate a toxin better than a weak or ill person.
- 6. **Dosage:** The effect of the poison depends upon its dosage. It is said that the dose determines whether a substance is a poison or remedy. A substance is usually considered a poison after a certain fixed quantity. Although this quantity is not fixed for all people, it is considered according to the average effect on the population. There are two considerable effects of poison on the body of a person; these are the subtle long-term chronic toxicity and immediate fatality.

Some poisons are lethal in microquantities, while others can affect in large doses. The significance of a dose can be understood by taking an example of a metal essential in the food, for example, iron, copper, manganese, zinc, etc.; if its dose is higher than the body requires, it can be lethal.

- Effective dose (ED): The effective dose is the quantity of a substance at which it shows its effect in the population. In most cases, ED50 is measured as a dose which induces a response in half of the targeted population.
- Lethal dose: The lethal dose (LD) 50 is the amount of drug which is expected to cause death of 50% population.
- 7. **Hypersensitivity:** It is basically the type of reaction initiated by the body against any other substances. Sometimes, it could be related to allergy. There is an assumption that hypersensitivity does not depend on wrong doses. Every person who is hypersensitive to a particular substance has a dose related that defines the quantity required to cause hypersensitivity to that person. The allergic response is actually a toxic response and can be sometimes fatal.
- 8. **Idiosyncrasy:** It is defined as a reaction produced by the body to a chemical genetically. It is a type of person that affects only those people who are genetically sensitized to that particular chemical or substance but will show no effect on others. In such cases, the person experiences discomfort for several hours or if the dose is high can be fatal also. For example- peanut allergy in some people.
- 9. **Tolerance:** It is the capability of a person to not produce any effect against a chemical that usually causes reaction to normal persons. It is a state of reduced or no reaction to a chemical. There are basically two types of mechanism that induces tolerance. First is when the toxin reaches the effective site, its quantity is very less. This is called dispositional tolerance. The second is because the tissues show reduced response to the toxin.

Tolerance can also be achieved if a drug is taken in a small quantity on a regular basis. This can be explained by taking the example of alcohol. When any human consume alcohol for the first time, he/she will show an effect even when the quantity is small, but eventually the effect will decrease and the person can tolerate a large amount also.

10. **Individual susceptibility:** It is defined as the different kinds of responses produced by different individuals to a particular harmful compound. It can be due to occupational or environmental factors and exposures. It is determined by complex genetic factors. Its effect depends upon the intensity of exposure. There is a gene uniqueness that varies from person to person; thus the same amount of exposure can show no effect in one individual, cause illness to other individual, and also could be fatal to someone as well.

#### 4. Route of administration of poison

The route of administration is the path through which a drug, toxin, or poison is taken or administered into the body of a person which is distinguished by the location where any drug is applied. It is mostly classified on the basis of its target:

- Topical—which has a local effect
- Enteral—which has a wide effect, i.e., affect the whole system
- Parental—which follows a systemic action

Poisons are given or taken so that death can occur at once by shock due to stoppage of body's vital systems. Drug addicts take drugs through inhalation or injection.

Route of administration plays a very important role in determination of death by poison as time in which death occurs are fastest in inhaled poisons, relatively slow in injected and lastly when ingested orally.

Some important features that are considered during the administration of poisons and can make a poison fatal are:

- Rate of dissolution of the poison that depends upon the physical form of the poison, i.e., gaseous, vapors, liquid, solid, etc.
- The surface area affected at the site of administration of the poison
- The circulation rate of blood in that route
- The solubility of the poison, i.e., lipid soluble or water soluble
- The concentration of the poison
- The time required by the poison to be absorbed completely from the site of administration

Routes of administration can be classified into two categories:

1. Enteral routes/gastrointestinal routes.

2. Parenteral routes.

**Enteral routes:** When the drug is administered through the gastrointestinal tract, it is defined as an enteral route. It has both oral and rectal routes. It also includes sublingual and sublabial routes. It is comparatively a slower mode of action for absorption of drugs:

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- Oral route: Generally absorption takes place in the tongue and the gums of the oral passage. The pH of the buccal cavity and mouth ranges from 4 to 5. Sublingual and supralingual routes have a significant role in absorption. The sublingual absorption is faster as the toxin is transformed directly to the heart, but it takes more time.
- **Rectal route:** Administration of drugs can be done through anus which directly absorbed in bloodstream through membrane of mucous. This administration can cause the burning of tissues or bleeding in rectum as the area is very sensitive.
- **Parental route:** It includes all the other routes that does not involve the gastrointestinal tract. It has a systemic effect on the body. It has the following categories of administration:
  - **Intradermal:** Here, the administration of drugs takes place from surface of skin. This type of poisoning is mostly found in chronic poisoning cases.
  - **Intravenous:** It is one of the fastest modes of drug administration as the injection is directly taken and the drug is transferred directly into the veins and thus is directly circulated into the blood quickly. Immediate death might be caused by this type of drug.
  - **Intraosseous:** It involves an administration of a drug directly into the bone marrow. This mode is actually used for administration of drugs for medical purposes.
  - **Intra-arterial:** It involves an administration of a drug into the artery directly through injection. It is a fast mode of administration.
  - **Intramuscular:** In this mode, the drug or poison is administered into the muscle of the thigh, upper arm, or buttock. The time required in this mode is greater than other parental modes.
  - **Subcutaneous:** In this mode, the drug is injected into the layer beneath the skin, i.e., the subcutaneous layer. The drug then goes to the small blood vessels and then to the bloodstream. This mode is used for mostly those protein drugs that would be destroyed if administered through the gastrointestinal tract.
  - **Inhalation:** In this mode, the nose is the primary path. Because of the presence of mucous membrane, the nasal aperture is very absorptive. The microparticles of poisons are easily absorbed and transported quickly to the lungs. From the lungs, they are circulated into the blood.

#### 5. Classification of poisons

Poisons are classified into two ways:

- i. Based on their action on the body.
- ii. Based on their physical and chemical properties [1].

Classification based upon the effect of poison on the body:

- 1. Corrosive: The poisons burn the tissues or organs when they come in contact with them, e.g.:
  - a. Strong acids such as H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, HCL, etc.
  - b. Strong alkalis such as hydroxides of Na, K, NH<sub>4</sub>, etc.

2. Irritants: The poisons irritate the tissues or organs when they come in contact with them [3]:

- a. Inorganic:
  - Nonmetallic phosphorous, chlorine, bromine, iodine, etc.
  - Metallic salts of arsenic, antimony, mercury, copper, lead, zinc, etc.

#### b. Organic:

- Vegetable—castor oil, madar, croton oil, etc.
- Animals—snake venom, cantharides, insect bites, etc.
- Mechanical—glass powder, needles, diamond dust, hair, etc.

3. Neurotics: Poisons affect the nervous system and the brain [3]:

- a. Cerebral:
  - Narcotic—opium and its alkaloids
  - Inebriant (depressant)—alcohol, ether, chloroform, and chloral hydrate
- b. Spinal:
  - Excitant (stimulants)—nux vomica and strychnine
  - Depressant—gelsemium
- c. Cardiorespiratory:
  - Cardiac—aconite, digitalis, oleander, and hydrocyanic acid (HCN)
  - Asphyxiants—carbon monoxide, carbon dioxide, and hydrogen sulfide
- 4. Miscellaneous: A number of chemicals having diverse actions on their body are included in this group [4]:
  - a. Animal poisons
  - b. Curare (an arrow poison)

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- c. Poisonous food articles
- d. Industrial poisons-methyl isocyanate (MIC)
- e. Fuels-petroleum and kerosene
- f. Insecticides—endrin, dichlorodiphenyltrichloroethane (DDT), and naphthalene
- g. Radioactive substances

Classification of poisons based upon their properties:

A. Inorganic poisons

- i. Metallic poisons:
  - a. Arsenic: It has been the most known and exclusively used throughout the ages to poison men and animals [1].

It is a white tasteless powder and a pinch of the poisons can kill two adult persons.

Arsenic for homicidal purposes is mixed with various food articles, e.g., cooked food, milk, tea, liquors, or medicines.

Arsenic in a metal form is not poisonous; its oxides are highly poisonous. It is extensively used in insecticides, etc. [5].

- b. Mercury: Chloride and nitrites of mercury are highly poisonous. They are used in chemical industry and as fungicides.
- c. Lead: Most of its compounds are poisonous. This is a slow poison, e.g., Sindoor adulterated with red lead oxide.
- d. Copper: Its salts are used in electroplating; copper sulfate is a poison.
- e. Thallium: Thallium salt is used as rat poison [6].
- f. Antimony: Its effect is like that of arsenic.
- ii. Nonmetallic poisons:
  - a. Cyanides: Cyanides of potassium and sodium are extremely poisonous, even in small quantities. They react with the acid of gastric juices in the stomach to form hydrocyanic acid, which paralyzes the respiratory center in the brain resulting in death due to respiratory failure [4].
  - b. Yellow phosphorus: In olden days it was used in match industry and several times proved highly poisonous.
  - c. Iodine: Only elemental iodine in high quantity is poisonous.

- d. Strong acids and alkalis: These are highly poisonous with corrosive effects, e.g., sulfuric acid, nitric acid, sodium, potassium hydroxides, etc.
- e. Gases: Phosphine gas kills rats when used on the rat holes and is poisonous for infants. MIC killed over 2000 persons and invalidated several others in a gas leak tragedy in Bhopal in 1984. Some other poisonous gases are HCN, carbon monoxide, hydrogen sulfide, arsine, etc. [3].

#### B. Organic poisons

i. Volatile poisons:

- a. Ethyl alcohol: It is poisonous if taken in excess.
- b. Other alcohols: Methyl alcohol and isopropyl alcohol are poisonous. Methanol, used in polish and chemical industries, is used in illicit liquor, and its intake causes paralysis, blindness, and death [3].
- c. Phenol: Phenol or carbolic acid could be poisonous. It is mostly used as a disinfectant [6].
- d. Miscellaneous substances: Various industrial chemicals like chlorinated hydrocarbons, benzene, chloral hydrate, etc. are poisonous. In several cases of poisoning, chloral hydrate could be used in illicit liquors.
- ii. Nonvolatile substances:
  - a. Alkaloids: Several narcotics and vegetable poisons contain alkaloids, e.g., strychnine, morphine, cocaine, nicotine, etc.
  - b. Barbiturates: These drugs are synthetic and induce sleep [1].
  - c. Glycosides: These drugs can cause cardiac arrest and could be fatal such as aconite, oleander digitalis, etc.
  - d. Insecticides and pesticides

**Poisoning:** It is known as the injurious effect caused by the action of a poison or a detrimental chemical substance. It leads to the development of adverse reaction toward the harmful chemicals or drugs. It is basically differentiated in three categories: suicidal, homicidal, and accidental. Cattle poisoning is the poisoning related to animals. Accidental poisoning is caused by negligence and carelessness. Homicidal poisoning includes the killing of a person due to the poison. Suicidal poisoning refers to the use of toxic chemicals in order to kill oneself.

Corrosive poisoning: It is caused by poisons such as acids and alkalis. They produce a corrosive action on the human body by causing ulcers and acute inflammation.

Metallic poisoning: Metals such as arsenic, mercury, lead, etc., when ingested, cause a deleterious effect. This is known as metallic poisoning.

Plant poison: The study of plant poisons is known as phytotoxicology. Plant poisons, or phytotoxins, comprise a vast range of biologically active chemical

substances, such as alkaloids, polypeptides, amines, glycosides, oxalates, resins, toxalbumins, etc.

#### 6. Alcohol

An alcohol is a drink that contains ethanol. Ethanol is made by fermentation of grains, fruits, and some resources of sugar. Chemically, it is a group of compounds whose saturated carbon chain has a "-OH" group. Alcohol is also a depressant, and in low dose, it can reduce tension, cause euphoria, and improve sociability, but in high dose it can cause stupor, drunkenness, and even death. Regular alcohol intake can cause cancer, alcoholism, dependency, etc. 33% of the total people in the world consumes alcohol. Drinks containing alcohol are broadly classified into three classes, i.e., beer, spirit, and wine, whose alcohol content varies between 3% and 50%. When diluted, alcohol has nearly sweet taste, but when concentrated it gives a burning sensation. 90% of the absorbed alcohol is metabolized by the liver and broken down into less toxic metabolites. Alcohol acts on the central nervous system (CNS) as a depressant on the cells of the cerebral cortex. Its adverse effects like a decrease in cognitive and psychomotive skills are well documented. Alcohol percentage (ABV) differs from one brand to another, for example, beers contain 5%, wines contain typically 13.5%, fortified wines contain 15–22%, spirits contain 30–40%, fruit juice contains less than 0.1%, and cider/wine coolers contain 4-8% ABV [1].

#### 7. Blood alcohol test

The goal of blood alcohol test is to check the concentration of alcohol in the body. This test result is known as blood alcohol concentration (BAC) which indicates alcohol % in the blood. It is directly proportional to the alcohol in the body, and alcohol hinders with people's decision, control on them and other characteristics [3]. This test can tell the presence of alcohol in blood for 12 hours [4]. Blood quickly absorbs alcohol and is measured within minutes of consuming alcoholic drink. The highest level of BAC result can be reached within an hour of consuming alcohol. Intake of food can vary the result. Liver breaks down almost 90% of alcohol and rest are given out from exhalation and urine [5].

#### 8. Sample collection

In case of deaths due to alcoholic intoxication, the viscera is collected and preserved in saturated saline. Preservation of sample is very important as if wrongly preserved it can ruin the examination. Generally, urine and blood are taken as samples.

A sterile needle must be cleaned up by the swab of a nonalcoholic disinfectant like aqueous mercuric chloride and aqueous benzalkonium chloride (Zephiran) before the suspect's skin is punctured with it. The use of an alcoholic disinfectant either may give false-positive results or may contribute to falsely high alcohol contents of blood. About 5–10 ml of the sample (blood) is taken in a test tube; an anticoagulant such as potassium oxide and EDTA and a preservative such as NaF are added and stored in the refrigerator at 40°C. The anticoagulant will prevent blood from clotting, and the preservative will inhibit the presence of microorganisms. The urine sample is also collected in the usual manner and preserved with 30 mg of phenyl mercuric nitrate for every 10 ml of urine [6].

#### 9. Extraction of ethyl alcohol from biological materials

Ethyl alcohol is isolated from biological materials by acid distillation. Viscera, vomit, stomach contents, and other materials should be analyzed separately. About 50–100 g of the viscera is taken and is finally minced by thin gruel and adding water (3–5 times) and sulfuric acid. It is passed to steam distillation which is generally heating it on the water bath. The condenser and the receiving flask should be well cooled with ice especially in the hot season, the outlet of the condenser being dipped in little water or NaOH solution. Some pieces of pumice stone are stored in the flask to avoid bumping. It is better to collect the distillate in 4–5 fractions, out of which the first one should not exceed 20 ml and the remaining fractions should be 50 ml each. The distillate contains alcohol and other volatile acids, etc. [6].

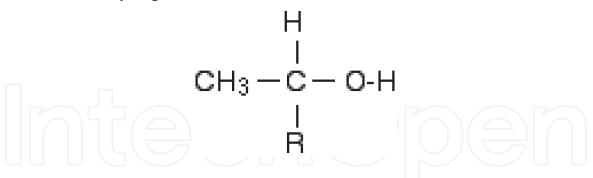
#### 10. Chemical analysis of ethyl alcohol

There are some tests which show the presence of ethyl alcohol in the exhibits.

#### 10.1 Iodoform test

Also known as triiodomethane reaction, it is used in the detection of  $CH_3CH$  (OH) which is present in alcohol. There are mainly two types of different mixtures used in this reaction which are mainly chemically equivalent. A pale yellow precipitate occurs if the result is positive [6].

In the above structure, "R" can be hydrogen or alkyl group or any other hydrocarbon group. In case when R denotes hydrogen, then the compound we have the possibility to find is primary alcohol ethanol. Ethanol is the only alcohol that gives an iodoform reaction. In case R is any hydrocarbon group, then it gives secondary alcohol groups. Tertiary alcohol is not able to contain R group because of the absence of hydrogen atom [7].



In 1 ml of distillate, a few drops of 10% NaOH are added dropwise till the solution becomes brown and warmed for a few minutes. A few drops of iodoform solution are added to change the color to yellow. The mixture has to be again heated on low flame/water bath; a yellow-colored precipitate is formed on standing. The precipitate has to be observed under a microscope. Characteristic hexagonal crystals of iodoform are seen which usually shows the presence of ethanol, acetaldehyde, isopropanol which on standing for long time breaks into flower like structure. This test initially involves oxidation followed by substitution and hydrolysis [6].

#### 10.2 Sulphomolybdic acid test

Add 1 gm of molybdic acid in 25 ml of a concentrated sulfuric acid which has the reagent. Mix 2 ml of this reagent when hot and with 2 ml of distillate. At the

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junction of both liquids, a ring will be formed which is deep blue in color. On shaking, the whole mixture will become deep blue which is due to ethyl alcohol. This test is very sensitive and it gives a negative result with acetone, acetaldehyde, and dilute solution of methyl alcohol. Only the strong solution of methyl alcohol gives a light blue color after several minutes [6].

#### 10.3 Ethyl benzoate test

Mix two drops of benzoyl chloride with 2 ml of the distillate. Add 10% of sodium hydroxide drop by drop till the solution becomes alkaline. By providing heat the irritating smell of benzoyl chloride will be replaced by sweet fruity odor of ethyl benzoate. Methyl alcohol gives this test also but not the iodoform test [6].

#### **11. Determination of ethyl alcohol in blood/urine**

In case of drunkenness, alcohol detection in the body is very important. Observing behavioral abnormalities of the suspect is the best method, but analyzing the breath, blood, and urine is the only way of confirming it. The analysis of breath alcohol can be performed on the spot with the help of breath-analyzer instruments like Alco-Sensor, Breathalyzer, etc. However, the alcohol content of the blood could be determined by using the modified version of the Kozelka and Hine/Cavett method [6].

In recent years, several methods in determining the alcohol in body fluids are described. Kent-Jones and Taylor reported the results of an investigation into the merits of two methods—the micro Cavett and that of Kozelka and Hine. The micro Cavett method is more accurate, but it suffered from serious inconsistencies in reproducibility, but the Kozelka and Hine method is less accurate and more timeconsuming but gives good reproducibility.

Nickolls modified the micro Cavett method which appears to give a more accurate result in comparison with the unmodified method. The simplicity of this procedure increases its use for routine work in laboratory [8].

#### 11.1 Cavett method/Kozelka and Hine method

The principle behind this method is the oxidation of alcohol, which is easy with acetic acid in the presence of oxidizing agents such as sulfuric acid and potassium dichromate. Reduction of each mL of N/20 potassium dichromate solution takes place that is equivalent to 0.575 mg of alcohol [6].

#### 11.2 Widmark's formula

This formula is used to estimate the amount in which alcohol is present in the body.

a. For blood analysis

$$a = cpr.$$

Here, a = Total amount of alcohol absorbed in the body; p = Weight of the person; c = Concentration of alcohol in the blood; r = Constant which is 0.5 in women and 0.68 in men

b. In urine analysis.

$$a = 3/4qpr.$$

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Here, a = Total alcohol content present in the body; p = Total weight of the person; q = Alcohol concentration in the urine; r = Constant, namely, 0.68 for men and 0.5 in women [6].

#### 12. Instrumental technique of analysis of ethyl alcohol

#### 12.1 Gas chromatography

There are several methods in determining ethanol in the blood, urine, and serum. One of the most important methods is gas chromatography (GC). The sample is injected in a heating chamber, and due to its high temperature, alcohol converts in vapors which are carried by inert carrier gas such as nitrogen through the column which is packed by an adsorbent material. Separation of different types of components depends on their different affinity, i.e., partition coefficient toward adsorbent phase which is stationary and later detected as shown in the figure below. A chromatogram so obtained helps in qualitative as well as quantitative analysis [6].

Various components of gas chromatography are [9]:

- Carrier gas
- Flow regulator
- Injector
- Column
- Stationary phase
- Oven
- Detectors
- Display device

The area covered by the peak represents the amount and position of a particular type of compound [6]. Operating conditions [10]:

Column: Porapak polymer bead 80–100 mesh or its equivalent, which can separate or resolve the ethanol. Column temperature: 1600°C. Carrier gas: Nitrogen. Rate of gas flow: 50 ml/minute. Detector: Flame ionization detector.

Alternative operating conditions:

**Column:** 0.3% Carbowax 20 M on 80–100 mesh Carbopak C, 2 m  $\times$  2 mm ID or its equivalent.

**Column temperature:** 350°C for 2 minutes and then programmed at 50°C per minute to 1750°C and hold for at least 8 minutes. **Carrier gas:** Nitrogen at 30 ml/minute [6].

#### 13. Conclusion

The purpose of this chapter is to discuss the mode of action and function of poisons once they reached in the human body. The impacts of poisons are severe and even cause death if not treated properly.

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