# PRACTICAL TOXICOLOGY

| أسم المادة | باللغة العربية | علم السموم | السنة الدراسية الثالثة | عدد الساعات الأسبوعية | | | |
|---|---|---|---|---|---|---|---|
| | باللغة الإنكليزية | Toxicology | | نظري | عملي | المجموع | عدد الوحدات |
| لغة التدريس للمادة - الإنكليزية | | | | 2 | 2 | 4 | 3 |

**The most important objectives of this course are:**

Students will be able to:

1- Understanding the toxicants in the environment, describe their route enter to the body.

2-Describe metabolic process, distribution, and excretion from the body.

3-Undertand the effect of toxicants in the living body.

4-Know the fundamental problems of toxicants in the world

5-Determine sources of toxicants

6-enhance their interest in gaining information about toxicants from websites

## Practical Syllabus of Toxicology

| No | Lab title |
|---|---|
| 1 | General introduction to practical toxicology. |
| 2 | Acute toxicity study, determination of LD50 |
| 3 | Drug toxicity on liver |
| 4 | Nicotine toxicity. |
| 5 | Metal toxicity |
| 6 | Culture media |
| 7 | Bacterial toxin |
| 8 | Fungal toxin |
| 9 | detection toxins in liquid material direct way |
| 10 | detection toxins in liquid material direct way |
| 11 | detection toxins in blood |
| 12 | toxicity of pesticides |
| 13 | toxicity of antibiotics |
| 14 | Air toxicant |
| 15 | Water toxicant |

L. 1

# General introduction to practical toxicology

## Learning objectives

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

- knowing the different terms in toxicology

❖ **Toxicology** is one of the oldest branches of pharmacology, and was once called or Toxicology knows poisons. Definition of Toxicology the definition of toxicology includes metabolism Excretion and method of action of these toxins Poisoning treatment.

❖ **Toxicology** is defined as the study of the adverse effects of chemicals on living organisms. The term toxicity is defined as the inherent capacity of a chemical to cause injury. Thus, all chemicals, including drugs, have some degree of toxicity.

❖ Toxicokinetic and toxicodynamic are two fundamental concepts in the field of toxicology.

❖ Toxicokinetic: They describe the processes by which toxic substances distributed, are absorbed, metabolized, eliminated in the body.

❖ Toxicokineticrefers to the study of the movement of toxic substances within the body. ◦Thisincludestheabsorption, distribution, metabolism, and excretion (ADME)of the toxicants.

❖ Toxicodynamic: They also describe how these substances exert their effects on target cells, tissues and Toxicodynamic involves understanding the mechanisms of action, dose response relationships, and factors influencing individual susceptibility to toxicity. organs.

❖ **Toxicity:** Toxicity is defined as the ability of a substance to cause harm to a living organism, and it is an estimated value expressed as the amount of poison per unit weight of the organism being treated with it.

# PRACTICAL TOXICOLOGY

- **Poison:** Poison was defined by orfila in 1821. any substance that enters the body of a living organism in any way and in a dose very small, causing a harmful effect on health or leading to totally dead.
- **Toxin:** A toxin is a toxic substance produced naturally by living organisms.
- **Toxicant:** A toxicant or poison is meant to be a toxic substance produced naturally or prepared by man.
- **Venom:** The expression venom means that it is the poison that is produced by the animal to be poisoned by it the degree of particularity other types of animals by means of a special mechanism designed to give venom.
- **Dose:** is the amount of the actual chemical compound that enters the body of the organism and expresses it milligrams of compound/kg of body weight(mg/kg

- **Dosage:** It is the amount of the chemical compound per unit weight of the exposed individual per day mg(dose)/kg(b.w.)/day.
- **LD50(LethalDose50):** It is the dose of a single substance that causes 50% of the population of living organisms (animals) to die as a result exposure to this substance by any method of exposure other than through breathing.
- **Less lethal dose low (LD**LO**):** LDLO is defined as the lowest dose of a substance which enters the body by any means of entry except through breathing, which causes death in human and animals.

# Acute toxicity

## Learning objectives

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

1. Mention introduction about acute toxicity.

2. illustrate the different time of toxicity exposures.

## Introduction

Acute toxicity can be defined as toxicity elicited immediately following short-term exposure to a chemical. By this definition, two components comprise acute toxicity: acute exposure and acute effect.

## ACUTE EXPOSURE AND EFFECT

In contrast to acute toxicity, chronic toxicity is characterized by prolonged exposure and sublethal effects elicited through mechanisms that are distinct from those that cause acute toxicity. Typically acute and chronic toxicity of a chemical are easily distinguished. For example, mortality occurring within two days of a single dose of a chemical would be a prime example of acute toxicity(figure 1).

1. Acute: Produced by a single dose or several small doses taken in a short period. Onset of symptoms is abrupt.
2. Chronic: Produced by small doses taken over a long period. Onset is insidious
3. Subacute: Characterised by a mixture of features of acute and chronic poisoning.

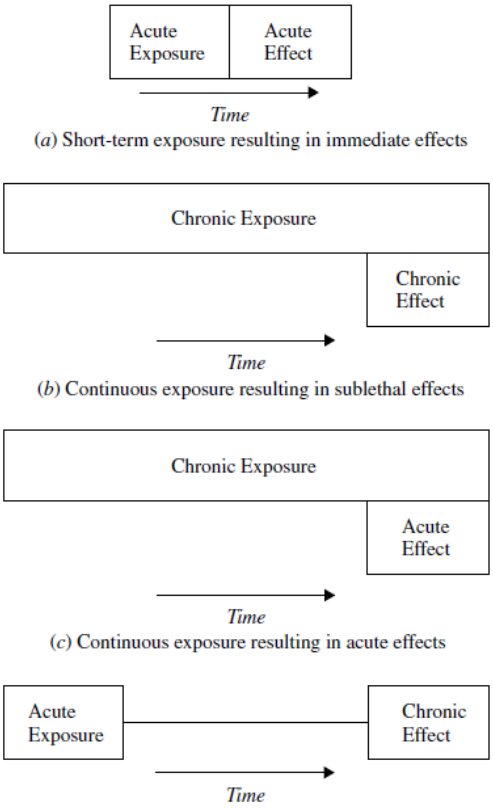


**Figure 1**. Examples of exposure/effect scenarios that result in either acute toxicity (a), chronic toxicity (b), or mixed acute/chronic toxicity (c,d). Examples for each scenario are provided in the text.

# PRACTICAL TOXICOLOGY

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## DRUG TOXICITY ON LIVER

**Learning objectives**

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

- Describe the different drugs that cause liver toxicity.

**Drug-induced liver injury (DILI)** is a cause of acute and chronic liver disease caused specifically by medications and the most common reason for a drug to be withdrawn from the market after approval.

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses (e.g. acetaminophen, paracetamol) and sometimes even when introduced within therapeutic ranges (e.g. halothane), may injure the organ.

### ❖ Drug metabolism in liver

Drug metabolism in liver: transferases are: glutathione, sulfate, acetate, glucoronic acid. P-450 is cytochrome P-450. Different pathways are shown for Drugs A, B and C. The human body subjects most, but not all, compounds to various chemical processes (i.e. metabolism) to make them suitable for elimination. This involves chemical transformations to (a) reduce fat solubility and (b) to change biological activity.

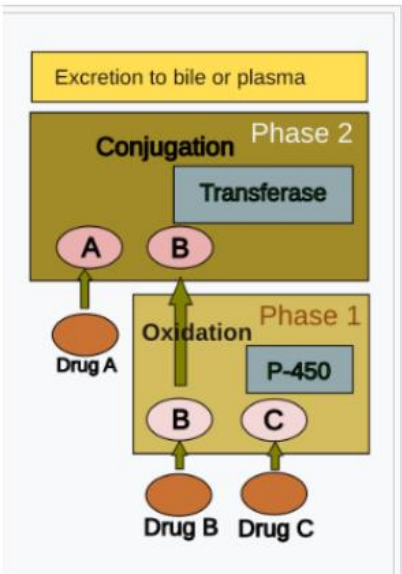


Figure. Drug Metabolism in liver

Drug metabolism is usually divided into two phases: phase 1 and phase 2. Phase 1 reaction is generally speaking to prepare a drug for phase 2. However, many compounds can be metabolized by phase 2 directly or be excreted without any phase 2 reactions occurring. Phase 1 reaction involves oxidation, reduction, hydrolysis, hydration and many other rare chemical reactions. These processes tend to increase water solubility of the drug and can generate metabolites that are more chemically active and/or potentially toxic. Most of phase 2 reactions take place in cytosol and involve conjugation with endogenous compounds via transferase enzymes. Phase 1 are typically more suitable for elimination.

- **Paracetamol**

Paracetamol also known as acetaminophen, and by the brand names of Tylenol and Panadol, is usually well-tolerated in prescribed dose, but overdose is the most common cause of drug-induced liver disease and acute liver failure worldwide. Damage to the liver is not due to the drug itself but to a toxic metabolite (N-acetyl-p-benzoquinone imine (NAPQI)) produced by cytochrome P-450 enzymes in the liver. In normal circumstances, this metabolite is detoxified by conjugating with glutathione in phase 2 reaction. In an overdose, a large amount of NAPQI is generated, which overwhelms the detoxification process and leads to liver cell damage. Nitric oxide also plays a role in inducing toxicity.

- **Nonsteroidal anti-inflammatory drugs**

Although individual analgesics rarely induce liver damage due to their widespread use, NSAIDs have emerged as a major group of drugs exhibiting hepatotoxicity. Both dose-dependent and idiosyncratic reactions have been documented. Aspirin and phenylbutazone are associated with intrinsic hepatotoxicity; idiosyncratic reaction has been associated with ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin. **LECTURE**

- **Glucocorticoids**

Glucocorticoids are so named due to their effect on the carbohydrate mechanism. They promote glycogen storage in the liver. An enlarged liver is a rare side-effect of long-term steroid use in children. The classical effect of prolonged use both in adult and paediatric population is steatosis.

- **Isoniazid**

Isoniazide (INH) is one of the most commonly used drugs for tuberculosis; it is associated with mild elevation of liver enzymes in up to 20% of patients and severe hepatotoxicity in 1-2% of patients.

# Nicotine toxicity

**Learning objectives**

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

- Demonstrate the introduction about nicotine toxicity.
- Understanding the Laboratory analysis used in exploring of nicotine toxicity.

## Introduction

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 nalysis (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 int**o**xication.

**-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.

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# Metal toxicity

**Learning objectives**

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

- Mention the Common Toxic Mechanisms and Sites of Action

## Common Toxic Mechanisms and Sites of Action

**Enzyme Inhibition/Activation:** A major site of toxic action for metals is interaction with enzymes, resulting in either enzyme inhibition or activation. **Two mechanisms are of particular importance:** inhibition may occur as a result of interaction between the metal and sulfhydryl (SH) groups on the enzyme, or **the metal** may displace an essential metal cofactor of the enzyme. For example, lead may displace zinc in the **zinc-dependent enzyme δ-aminolevulinic acid dehydratase (ALAD)**, thereby inhibiting the synthesis of heme, an important component of hemoglobin and heme-containing enzymes, such as cytochromes.

**Subcellular Organelles:**Toxic metals may disrupt the structure and function of a number of organelles. For example, enzymes associated with the endoplasmic reticulum may be inhibited, metals may be accumulated in the lysosomes, respiratory enzymes in the mitochondria may be inhibited, and metal inclusion bodies(fig. 2) may be formed in the nucleus.

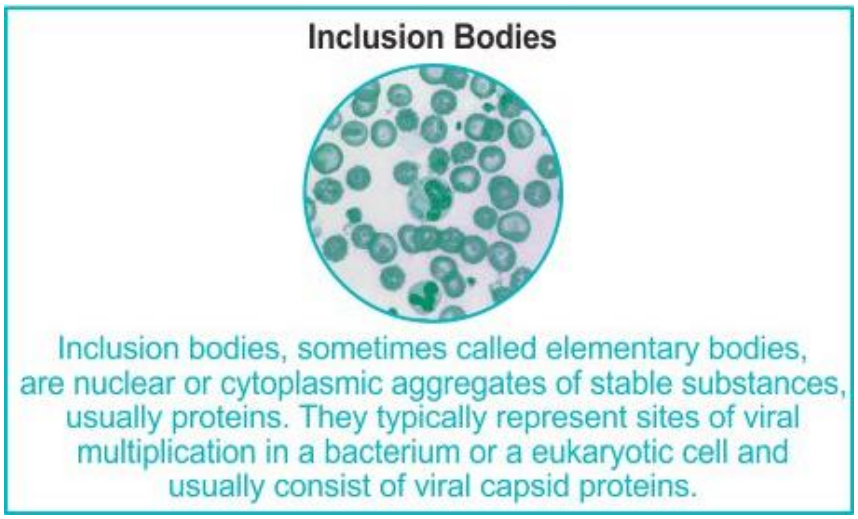


Figure 2: Inclusion bodies

**Carcinogenicity:** A number of metals have been shown to be carcinogenic in humans or animals. **Arsenic, certain chromium** compounds, and nickel are known human carcinogens; beryllium, cadmium, and cisplatin are probable human carcinogens. The carcinogenic action, in some cases, is thought to result from the interaction of the metallic ions with DNA.

**Kidney:** Because the kidney is the main excretory organ of the body, it is a common target organ for **metal toxicity**. **Cadmium and mercury**, in particular, are potent **nephrotoxicants**.

**Nervous System:** The nervous system is also a common target of toxic metals; particularly, **organic metal compounds**. For example, **methylmercury**, **because it is lipid soluble, readily crosses the blood-brain barrier and enters the nervous system**. By contrast, **inorganic mercury compounds**, which are **more water soluble, are less likely to enter the nervous system and are primarily nephrotoxicants**. Likewise organic lead compounds are mainly neurotoxicants, whereas the first site of **inorganic lead** is enzyme inhibition (e.g., enzymes involved in heme synthesis).

**Endocrine and Reproductive Effects:** Because the male and female reproductive organs are under complex neuroendocrine and hormonal control, any toxicant that alters any of these processes can affect the reproductive system. In addition metals can act directly on the sex organs. **Cadmium** is known to produce testicular injury after acute exposure, and lead accumulation in the testes is associated with **testicular degeneration**, inhibition of **spermatogenesis, and Leydig-cell atrophy**(fig. 3).

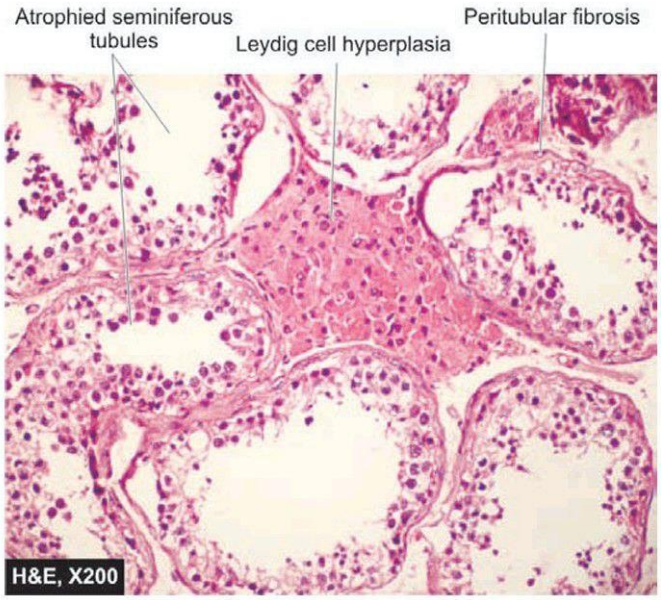


Figure 3: Histopathological changes of Leydig-cell

**Respiratory System:** Occupational exposure to metals in the form of metal dust makes the respiratory system a likely target. **Acute exposure may cause irritations and inflammation of the respiratory tract, whereas chronic exposure may result in fibrosis (aluminum) or carcinogenesis (arsenic, chromium, nickel).**

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# Culture media

**Learning objectives**

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

- **Understanding the cell culture techniques**

## CELL CULTURE TECHNIQUES

While scientists have had the ability to culture many unicellular organisms for some time, recent advances in the culture of cells from multicellular organisms have played a pivotal role in recent advances in toxicology. Cells can be isolated and either maintained in a viable state for enough time to conduct informative experiments or, in some cases, propagated in culture. The advantages of cultured cells are that they can provide living systems for the investigation of toxicity that are simplified relative to the intact organism and they can be used as replacements for whole animal toxicity testing if the toxic end point can be validated. Human cells play an important role in the extrapolation of toxic effects, discovered in experimental animals, to humans. Cultured cells, from humans or other mammals, are utilized in many of the molecular methods mentioned below. There are, however, limitations in the use of cellular methods. It has not been possible to culture many cell types, and of those that have been cultured, the loss of differentiated cell function is a common problem. Extrapolation of findings to the intact animal is often problematical and the use of undefined media constituents such as serum, often essential for cell viability, may have unwanted or undefined effects on bcell function and toxicant bioavailability.

- **Suspension Cell Culture**

Circulating blood cells or cells easily obtained by lavage such as peritoneal and alveolar macrophages can normally survive in suspension culture when provided with a suitable nutrient medium. Cells from organized solid organs or tissues must be separated from the tissue and, if possible, separated into cell types, before being suspended in such a medium. Cell association within organs depends on protein complex formation, which in turn is Ca2+ dependent. Consequently dissociation media generally contain a proteolytic enzyme and the Ca2+ chelator EDTA. There are a number of methods available to separate cell types from the mixture of dispersed cells, the commonest being centrifugation without a density gradient, wherein cells are separated by size, or centrifugation through a density gradient wherein cells are separated on the basis of their buoyant density. Cells in suspension may be maintained for a limited period of time in defined media or for longer periods in nutrient, but less well-defined, media. In either case these cultures are often used for studies of xenobiotic metabolism.

### ❖ Monolayer Cell Culture

Proliferation of most cells in culture requires attachment to a substrate and occurs until limited by cell-to-cell contact, resulting in the formation of a cellular monolayer. The substrate provided for attachment is usually polystyrene modified to carry a charge. The medium for continued maintenance and growth contains salts and glucose, usually with a bicarbonate buffer. Because of the bicarbonate buffering system these cultures are maintained in a 5–10% CO2 atmosphere in a temperature and humidity controlled incubator. Many cells require serum for optimal growth, inducing considerable variability into the experimental system. Since the factors provided by serum are numerous and complex, defined serum substitutes are not always successful. The factors provided by serum include proteins such as growth factors, insulin and transferrin (to provide available iron), small organic molecules such as ethanolamine, and pyruvate and inorganic ions, such as selenium.

### Indicators of Toxicity in Cultured Cells

Routine observation of cultured is usually carried out by phase contrast microscopy, utilizing the inverted phase contrast microscope. More recently, more detailed observations have become possible utilizing fluorescent tags and inverted fluorescent microscopes. Fluorescent tags currently in use permit the assessment of oxidant status and mitochondrial function as well as the

intracellular concentration of sulfhydryl groups, Ca2+, H+, Na+, and K.+ Toxicity to cultured cells may be the result either of inadequacies in the culture or the toxicity effects of the chemical being investigated. Short-term toxicity is usuallyevaluated by examination of end points that indicate effects on cellular organelles such as leakage of cell constituents into the medium, uptake of dyes into the cell and the formation of surface "blebs." This is illustrated in (Figure 4). Longer term assessments of cell toxicity are highly dependent on the relevant toxic end point. They may include measurement of growth competence, apoptosis, and/or necrosis, incorporation of radioactive precursors into essential cellular constituents such as RNA, DNA, and protein and specialized cellular functions. Some examples of the use of cultured cell lines in the study of toxicity effects are shown in (Table 1).

Table 1. Examples of Application of Cell Lines Retaining Differentiated Properties in the Study of Toxic Effects∗

| Cell Line | Source | Differentiated Cell Type | Toxicant | Measured End Point |
|---|---|---|---|---|
| N1E-115 | Mouse neuroblastoma | Cholinergic neuron | Lead<br>Pyrethroid insecticide | Blockage of voltage-dependent $Ca^{2+}$ channels<br>Prolonged open time for voltage-dependent $Na^{+}$ channels |
| PC12 | Rat pheochromocytoma (adrenal medullary tumor) | Adrenergic neuron | Tricresyl phosphate (organophosphate) | Inhibition of neurofilament assembly and axonal growth |
| SK-N-S11 | Human neuroblastoma | Neuron | $N_2O$ (anesthetic) | Depressed cholinergic $Ca^{2+}$ signaling |
| Hepa-1 | Mouse hepatoma | Hepatocyte | 2,3,7,8-terachloro-dibenzodioxane (TCCD) | Induction of CYP1A1 and 1B1 |
| H114 E | Rat hepatoma | Hepatocyte | Polychlorinated biphenyls (PCBs) | Induction of CYP1A1 |
| HepG2 | Human hepatoblastoma | Hepatocyte | Cyclophosphamide (antineoplastic) | Cytochrome P450-dependent genotoxicity |
| 3T3-L1 | Mouse embryo fibroblasts | Adipocytes | TCDD | Inhibition of glucose transport and lipoprotein lipase |
| Y1 | Mouse adrenocortical tumor | Adrenocortical cell | Methyl sulfone metabolites of DDT and PCBs | Inhibition of corticosterone synthesis by competitive inhibition of cytochrome P450 |
| LLC-PK1 | Pig kidney | Renal tubule epithelial cell | Cadmium | Cytotoxicity, apoptosis |
| MDCK | Dog kidney | Renal tubule epithelial cell | Organic mercury compounds | Cytotoxicity, transepithelial leakiness |

*Source*: E. Hodgson and R. C. Smart, eds., *An Introduction to Modern Toxicology*, 3rd ed. New York: Wiley, 2001.

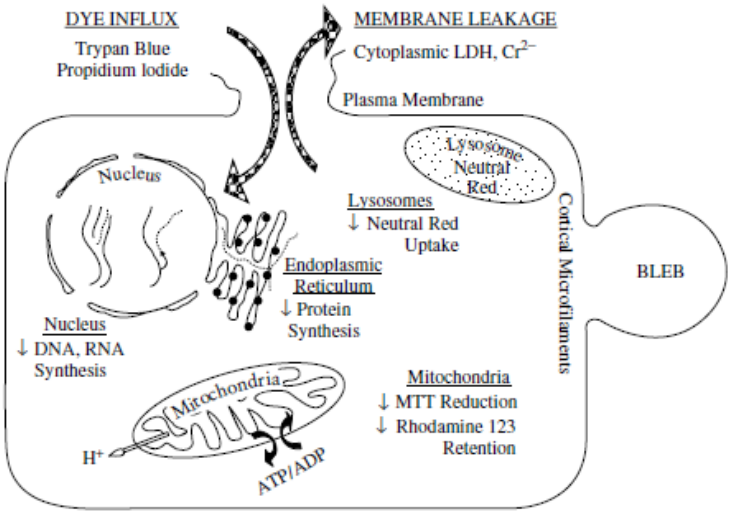

Figure 4. Idealized diagram of a cell to illustrate parameters often used to measure cytotoxicity and the corresponding affected subcellular organelle.

L. 7

# TOXINS
# MICROBIAL TOXINS

**Learning objectives**

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

- mention the bacterial toxins

## Bacterial toxins

The term “microbial toxin” is usually reserved by microbiologists for toxic substances produced by microorganisms that are of high molecular weight and have antigenic properties; toxic compounds produced by bacteria that do not fit these criteria are referred to simply as poisons. Many of the former are proteins or mucoproteins and may have a variety of enzymatic properties. They include some of the most toxic substances known, such as tetanus toxin, botulinus toxin(fig. 5), and diphtheria toxin. Bacterial toxins may be extremely toxic to mammals and may affect a variety of organ systems، including the nervous system and the cardiovascular system. A detailed account of their chemical nature and mode of action is beyond the scope of this volume. The range of poisonous chemicals produced by bacteria is also large. Again, such compounds may also be used for beneficial purposes, for example, the insecticidal properties of Bacillus thuringiensis, due to a toxin, have been utilized in agriculture for some time.

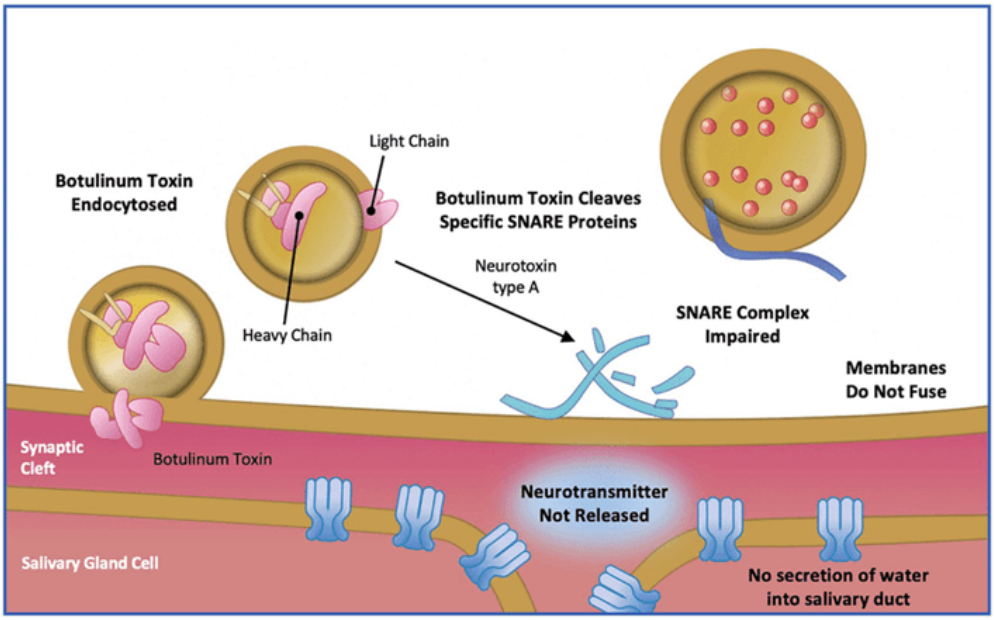


**Figure 5.** Botulinus toxin

# FUNGAL TOXINS

L. 8

**Learning objectives**

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

- See the fungal toxins

## Mycotoxins

Mycotoxins do not constitute a separate chemical category, and they lack common molecular features. Mycotoxins of most interest are those found in human food or in the feed of domestic animals. They include the ergot alkaloids(fig. 6) produced by Claviceps sp., aflatoxins (fig. 7)and related compounds produced by Aspergillus sp., and the tricothecenes produced by several genera of fungi imperfecti, primarily Fusarium sp. The ergot alkaloids are known to affect the nervous system and to be vasoconstrictors.

**Figue 6.** Ergot alkaloids

**Figure 7.** Aflatoxins

Historically they have been implicated in epidemics of both gangrenous and convulsive ergotism (St. Anthony's fire), although such epidemics no longer occur in humans due to increased knowledge of the cause and to more varied modern diets. Outbreaks of ergotism in livestock do still occur frequently, however. These compounds have also been used as abortifacients. The ergot

alkaloids are derivatives of ergotin, the most active being, more specifically, amides of lysergic acid. Aflatoxins are products of species of the genus Aspergillus, particularly A flavus‹ a common fungus found as a contaminant of grain, maize, peanuts, and so on. First implicated in poultry diseases such as Turkey-X disease, they were subsequently shown to cause cancer in experimental animals and, from epidemiological studies, in humans. Aflatoxin B1, the most toxic of the aflatoxins, must be activated enzymatically to exert its carcinogenic effect. Tricothecenes are a large class of sesquiterpenoid fungal metabolites produced particularly by members of the genera Fusarium and Tricoderma. They are frequently acutely toxic, displaying bactericidal, fungicidal, and insecticidal activity, as well as causing various clinical symptoms in mammals, including diarrhea, anorexia, and ataxia. They have been implicated in natural intoxications in both humans and animals, such as Abakabi disease in Japan and Stachybotryotoxicosis in the former USSR, and are the center of a continuing controversy concerning their possible use as chemical warfare agents. Mycotoxins may also be used for beneficial purposes. The mycotoxin avermectin is currently generating considerable interest both as an insecticide and for the control of nematode parasites of domestic animals.

# DETECTION TOXINS



## Learning objectives

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

- Explain the Detection toxins

## 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 ofanalytical 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

**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 pointof- 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, an  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.

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# DETECTION TOXINS IN BLOOD

## Learning objectives

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

- **Understand the General laboratory tests in clinical toxicology used in detection toxins in blood.**

## General laboratory tests in clinical toxicology

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

## A. Biochemical tests

### Blood glucose:

Determination of blood glucose is essential to know those toxic substances that affect blood glucose biotransformation. A toxicant that causes hypoglycemia includes insulin, iron, acetyl salicylic acid & so on. Hyperglycemia is a less common complication of poisoning than hypoglycemia, but has been reported after over dosage with acetylsalicylic acid, salbutamol and theophylline. Electrolytes, blood gases and pH Toxic substances or their metabolites, which inhibit key steps in intermediary biotransformation, are likely to cause metabolic acidosis owing to the accumulation of organic acids, notably lactate. Measurement of the serum or plasma anion gap can be helpful. The anion gap is usually calculated as the difference between the sum of sodium & potassium concentration and the sum of the chloride and bicarbonate concentrations ((Na++k+) + (Cl- + HCO3.( It is normally about 10mmol/l.

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

### Plasma enzymes

The plasma activities of liver enzymes, such as aspartate aminotransferase, alanine aminotransferase may increase rapidly after absorption of toxic doses of

substances that can cause liver necrosis, notably paracetamol, carbon tetrachloride, and copper salts.

**Cholinesterase activity**

Plasma cholinesterase is a useful indicator of exposure to organophosphorus compounds or carbamates, and a normal plasma cholinesterase activity effectively excludes acute poisoning by these compounds. The diagnosis can sometimes be assisted by detection of a poison or metabolite in a body fluid, but the simplest method available is relatively insensitive.

**Measurement of serum osmolality**

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

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 antiinflammatory 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.

# TOXICITY OF PESTICIDES

L. 11

## Learning objectives

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

- Describe the different pesticides that cause toxicity in environment.

**The toxicity of a pesticide** is its capacity or ability to cause injury or illness. The toxicity of a particular pesticide is determined by subjecting test animals to varying dosages of the active ingredient (a.i.) and each of its formulated products. The active ingredient is the chemical component in the pesticide product that controls the pest. The two types of toxicity are acute and chronic.

**Acute toxicity of a pesticide** refers to the chemical's ability to cause injury to a person or animal from a single exposure, generally of short duration. The four routes of exposure are dermal (skin), inhalation (lungs), oral (mouth), and eyes. Acute toxicity is determined by examining the dermal toxicity, inhalation toxicity, and oral toxicity of test animals. In addition, eye and skin irritation are also examined.

**Acute toxicity is measured** as the amount or concentration of a toxicant-- the a.i.--required to kill 50 percent of the animals in a test population. This measure is usually expressed as $LD_{50}$ (lethal dose 50) or $LC_{50}$ (lethal concentration 50). Additionally, the $LD_{50}$ and $LC_{50}$ values are based on a single dosage and are recorded in milligrams of pesticide per kilogram of body weight (mg/kg) of the test animal or in parts per million (ppm). $LD_{50}$ and $LC_{50}$ values are useful in comparing the toxicities of different active ingredients and different formulations containing the same active ingredient. *The lower the $LD_{50}$ or $LC_{50}$ of a pesticide product, the greater its toxicity to humans and animals.* Pesticides with a high $LD_{50}$ are the least toxic to humans if used according to the directions on the product label.

**The chronic toxicity of a pesticide** is determined by subjecting test animals to long-term exposure to the active ingredient. Any harmful effects that occur from small doses repeated over a period of time are termed chronic effects. Some of the suspected chronic effects from exposure to certain pesticides include birth defects, production of tumors, blood disorders, and neurotoxic effects (nerve disorders). The chronic toxicity of a pesticide is more difficult to determine through laboratory analysis than acute toxicity.

**Products are categorized** on the basis of their relative acute toxicity (their $LD_{50}$ or $LC_{50}$ values). Pesticides that are classified as highly toxic (Toxicity Category I) on the basis of either oral, dermal, or inhalation toxicity must have the signal words DANGER and POISON printed in red with a skull and crossbones symbol prominently displayed on the front panel of the package label. The Spanish equivalent for DANGER, "PELIGRO," must also appear on the labels of highly toxic chemicals. The acute (single dosage) oral $LD_{50}$ for pesticide products in this group ranges from a trace amount to 50 mg/kg. For example, exposure of a few drops of a material taken orally could be fatal to a 150-pound person.

Some pesticide products have the signal word **DANGER** without the skull and crossbones symbol. This is because possible skin and eye effects are more severe than suggested by the acute toxicity ($LD_{50}$) of the product.

Pesticide products considered moderately toxic (Toxicity Category II) must have the signal word WARNING and "AVISO" (the Spanish equivalent) displayed on the product label. In this category, the acute oral $LD_{50}$ ranges from 50 to 500 mg/kg. A teaspoon to an ounce of this material could be fatal to a 150-pound person.

**Pesticide products classified** as either slightly toxic or relatively nontoxic (Toxicity Categories III and IV) are required to have the signal word CAUTION on the pesticide label. Acute oral $LD_{50}$ values in this group are greater than 500 mg/kg. An ounce or more of this material could be fatal to a 150-pound person.

# PRACTICAL TOXICOLOGY

Despite the fact that some pesticide products are considered only slightly toxic or relatively nontoxic, all pesticides can be hazardous to humans, animals, other organisms, and the environment if the instructions on the product label are not followed. Use the pesticide only as recommended by the manufacturer. As the applicator, you are legally responsible for any misuse of a pesticide.

Table 2 summarizes the $LD_{50}$ and $LC_{50}$ values for each route of exposure for the four toxicity categories and their associated signal word. For example, an active ingredient with a dermal $LD_{50}$ of 1,000 mg/kg would be in Toxicity Category II with a WARNING signal word. Keep in mind, an active ingredient may have a high $LD_{50}$ placing it in a Toxicity Category II, III, or IV but also have corrosive eye/skin effects t hat take priority and place it in Toxicity Category I.

**Table 2.** Toxicity Categories for Active Ingredients

| Routes of Exposure | Toxicity Cat. I | Toxicity Cat. II | Toxicity Cat. III | Toxicity Cat. IV |
|---|---|---|---|---|
| Oral $LD_{50}$ | Up to and including 50 mg/kg | 50-500 mg/kg | 500-5,000 mg/kg | >5,000 mg/kg |
| Inhalation $LC_{50}$ | Up to and including 0.2 mg/l | 0.2-2 mg/l | 2-20 mg/l | >20 mg/l |
| Dermal $LD_{50}$ | Up to and including 200 mg/kg | 200-2,000 mg/kg | 2,000-20,000 mg/kg | >20,000 mg/kg |
| Eye Effects | Corrosive corneal opacity not reversible within 7 days | Corneal opacity reversible within 7 days; irritation persisting for 7 days | No corneal opacity; irritation reversible within 7 days | No irritation |
| Skin Effects | Corrosive | Severe irritation at 72 hours | Moderate irritation at 72 hours | Mild or slight irritation at 72 hours |
| Signal Word | DANGER POISON | WARNING | CAUTION | CAUTION |

Although every pesticide is different and the product label should be consulted to determine the personal protective equipment (PPE) requirements for each chemical,

some general rules apply for choosing PPE according to the different toxicity categories (Table 3). The acute oral and dermal $LD_{50}$ values of commonly used pesticides are listed in the following tables and include acaricides, bactericides, fungicides, herbicides, insect growth regulators, insecticides, nematicides, and plant growth regulators. The common chemical name of the active ingredient followed by an example of a trade name is listed in the first column. Use categories (general or restricted) are indicated in the second column. The acute oral $LD_{50}$ and acute dermal $LD_{50}$ are in the third and fourth columns. The fifth column indicates the restricted-entry interval (REI). The REI is the time immediately after a pesticide application when entry into the treated area is limited.

**Table 3.** Minimum PPE and Work Clothing for Pesticide-Handling Activities

| Route of Exposure | Toxicity Cat. I | Toxicity Cat. II | Toxicity Cat. III | Toxicity Cat. IV |
|---|---|---|---|---|
| Dermal toxicity or skin irritation potential | Coveralls worn over long-sleeved shirt and long pants<br><br>Socks<br>Chemical-resistant footwear<br>Chemical-resistant gloves | Coveralls worn over short-sleeved shirt and short pants<br>Socks<br>Chemical-resistant footwear<br>Chemical-resistant gloves | long-sleeved shirt and long pants<br><br>Socks<br>Chemical-resistant footwear<br>no minimum | long-sleeved shirt and long pants<br><br>Socks<br>Chemical-resistant footwear<br>no minimum |
| Inhalation toxicity | Respiratory protection device | Respiratory protection device | no minimum | no minimum |
| Eye irritation potential | Protective eyewear | Protective eyewear | no minimum | no minimum |

# PRACTICAL TOXICOLOGY

## TOXICITY OF ANTIBIOTICS

L. 12

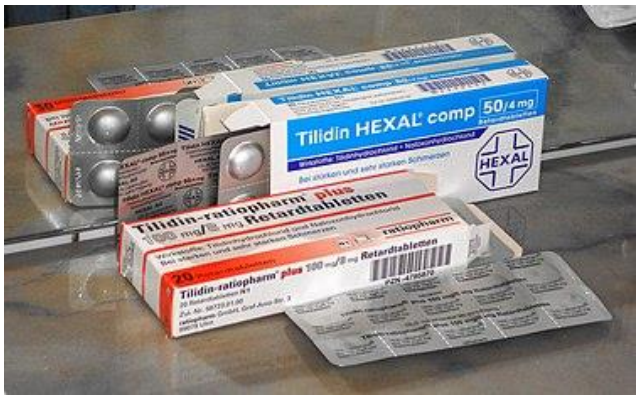
### Learning objectives

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

- Mention introduction about Antibiotics
- Describe the toxicity of different antibiotics

### ANTIBIOTIC

An **antibiotic** is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections. They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity. Antibiotics are not effective against viruses such as the ones which cause the common cold or influenza.[5] Drugs which inhibit growth of viruses are termed antiviral drugs or antivirals. Antibiotics are also not effective against fungi. Drugs which inhibit growth of fungi are called antifungal drugs.




### Side effects

Health advocacy messages such as this one encourage patients to talk with their doctor about safety in using antibiotics.

Antibiotics are screened for any negative effects before their approval for clinical use, and are usually considered safe and well tolerated. However, some antibiotics have been associated with a wide extent of adverse side

effects ranging from mild to very severe depending on the type of antibiotic used, the microbes targeted, and the individual patient. Side effects may reflect the pharmacological or toxicological properties of the antibiotic or may involve hypersensitivity or allergic reactions(fig. ). Adverse effects range from fever and nausea to major allergic reactions, including photodermatitis(fig. ) and anaphylaxis.

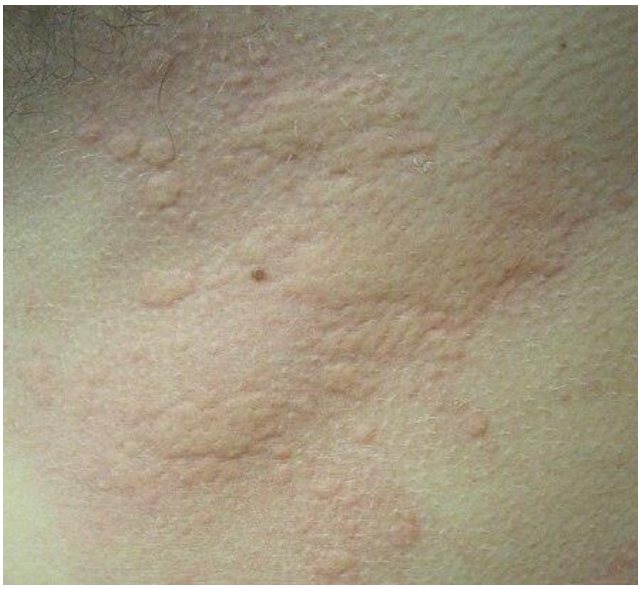


Figure . Allergic reaction.

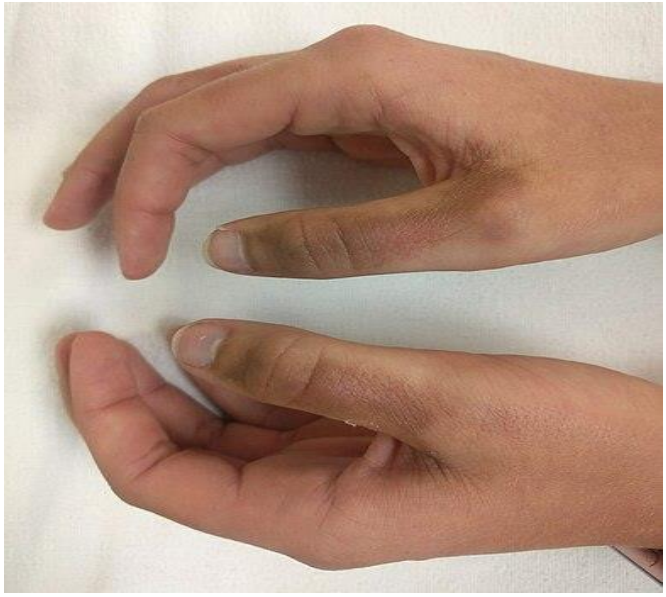


Figure . Photodermatitis

Common side effects of oral antibiotics include diarrhea, resulting from disruption of the species composition in the intestinal flora, resulting, for example, in overgrowth of pathogenic bacteria, such as *Clostridioides difficile*. Taking probiotics during the course of antibiotic treatment can help prevent antibiotic-associated diarrhea. Antibacterials can also affect the vaginal flora, and may lead to overgrowth of yeast species of the genus *Candida* in the vulvo-vaginal area.[50] Additional side effects can result from interaction with other

drugs, such as the possibility of tendon damage from the administration of a quinolone antibiotic with a systemic corticosteroid.

Some antibiotics may also damage the mitochondrion, a bacteria-derived organelle found in eukaryotic, including human, cells.[52] Mitochondrial damage cause oxidative stress in cells and has been suggested as a mechanism for side effects from fluoroquinolones. They are also known to affect chloroplasts.

L. 13

# AIR TOXICANT

## Learning objectives

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

- **Understanding the air pollutions, sources, and air pollutants.**

## Air pollutant

**Air pollution** is the presence of substances in the atmosphere that are harmful to humans and other living beings, or cause damage to the environment. Air pollution can be chemical, physical or biological(fig. ).

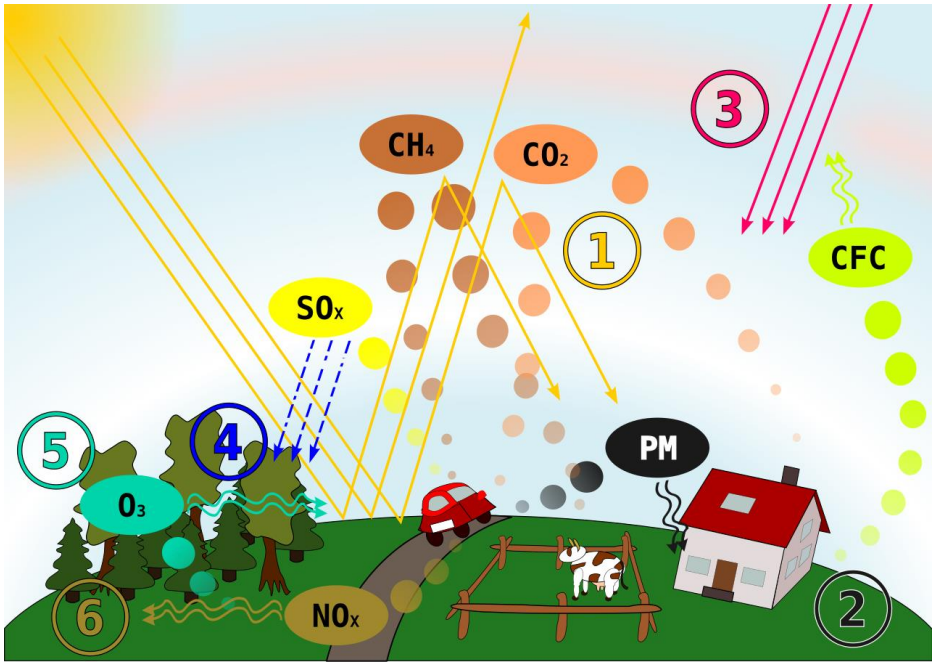



**Figure 8.** Air pollutant

## Sources of air pollution

There are many different sources of air pollution. Some air pollutants (such as **nitrogen oxides**) originate mainly from **human activities**, while some (notably **radon gas**) come mostly from **natural sources**. However, many air pollutants (including dust and **sulfur dioxide**) come from a mixture of natural and human sources.

**Human sources** edit

Most of the world's air pollution is from burning fossil fuels for industry, construction, transportation, and heating, although humans make air pollution in many other ways. For instance, nuclear weapons(Figure 9), toxic gases, germ warfare, and rocketry can cause air pollution(figure 10 a, b).

Figure 9. Demolition of the cooling towers of a power station, Athlone, Cape Town, South Africa, 2010

Figur 10a. Controlled burning

Figure 10 b. Smoking of fish over an open fire in Ghana, 2018

cles increases

**Industry and construction**

The burning of fuels to produce electricity causes air pollution. Lignite and coal produce most air pollution, followed by oil. The burning of fossil gas and biomass causes less air pollution. Methane leaks are common in oil and gas production.

**Major pollutants**

An air pollutant is a material in the air that can have many effects on humans and the ecosystem. The substance can be solid particles, liquid droplets, or gases, and often takes the form of an aerosol (solid particles or liquid droplets dispersed and carried by a gas). A pollutant can be of human or natural origin.

Pollutants are classified as **primary or secondary**. **Primary pollutants** are produced directly by a source and remain in the same chemical form after they have been emitted into the atmosphere. Examples include ash from **a volcanic eruption**, **carbon monoxide gas from motor vehicle exhausts, and sulfur**

**dioxide** released from factories. **Secondary pollutants** are **not emitted directly**. Rather, they form in the air when primary pollutants react or interact. Ground-level ozone is a prominent example of a secondary pollutant. Some pollutants may be both primary and secondary: they are both emitted directly and formed from other primary pollutants.

## Ammonia

**Ammonia ($NH_3$) is emitted mainly by agricultural waste**. It is normally encountered as a gas with a characteristic pungent odor. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to foodstuffs and fertilizers. Although in wide use, ammonia is both caustic and hazardous. In the atmosphere, ammonia reacts with oxides of nitrogen and sulfur to form secondary particles.

## Carbon dioxide

**Carbon dioxide ($CO_2$) is mainly emitted by the burning of fossil fuels**. It is potentially lethal at very high concentrations (typically 100 times "normal" atmospheric levels). Although the World Health Organization recognizes $CO_2$ as a climate pollutant, it does not include the gas in its *Air Quality Guidelines* or set recommended targets for it. Workplace exposure limits exist in places like UK (5,000 ppm for long-term exposure and 15,000 ppm for short-term exposure). Natural disasters like the limnic eruption at Lake Nyos can result in a large sudden release as well.

## Carbon monoxide

Carbon monoxide (CO) is a colorless, odorless, toxic gas. **It is a product of combustion of fuel such as natural gas, coal or wood**. In the past, emissions from vehicles were the main source of CO, but modern vehicles do not emit much CO. Now, wildfires and bonfires are the main source of outdoors

CO. Indoors, CO is a larger problem and mainly comes from cooking and heating.

**Nitrogen oxides**

Nitrogen oxides ($NO_x$), particularly **nitrous oxide (NO), are mostly created by the burning of fossil fuels, and in lesser amounts by lightning**. Nitrogen dioxide ($NO_2$) is formed from NO in a reaction with other atmospheric gases. NO and $NO_2$ can form acid rain, can form into a haze, and can cause nutrient pollution in water. $NO_2$ is a reddish-brown toxic gas with a strong odor, whereas NO is odorless and does not have a color.

**Sulfur dioxide**

**Sulfur dioxide ($SO_2$) is produced by volcanoes and in various industrial processes.** Coal and petroleum often contain sulfur compounds, and their combustion generates sulfur dioxide. High concentrations of SO2 in the air upon emissions generally also lead to the formation of other sulfur oxides (SOx). SOx can react with other compounds in the atmosphere to form small particles and contribute to particulate matter (PM) pollution. At high concentrations, gaseous SOx can harm plants by damaging foliage and decreasing growth. SO2 and other sulfur oxides can contribute to acid rain. Further oxidation of $SO_2$, usually in the presence of a catalyst such as $NO_2$, forms $H_2SO_4$, and thus acid rain is formed.

# WATER TOXICANT

L. 14

## Learning objectives

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

- Mention the introduction about water toxicants
- Describe the types of toxicants
- Understanding the laboratory analysis used in exploring aaboue water toxicants.

## water tocicants

## 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.

### ❖ 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 ingested absorb about10% of the amount while young children absorb closer to .%50The 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 halflife 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)
- 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).

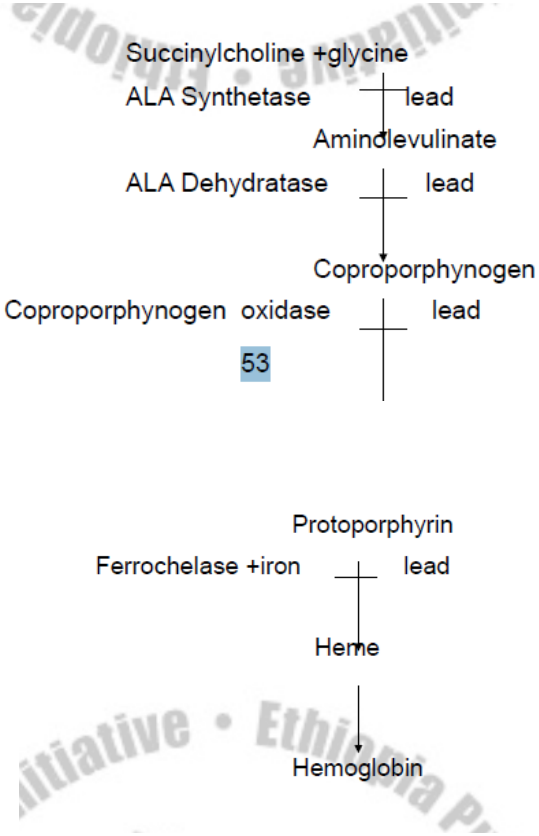



Fig. Lead interference with the biosynthesis of heme The sign and symptoms of lead poisoning may include anorexia, apathy, behavioral changes, persistent vomiting, convulsions (acute poisoning) & ataxia, wrist & ankle drop, chronic nephritis (chronic poisoning)

## Laboratory findings

### A. Complete blood count

1-Anemia –- Hemoglobin level of less than 10gm/dl can be seen

2-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.

3 -Eosinophilia – common finding but non specific

4 -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 i n h i b i t i o n o f t h e enzyme

coproporphyrinogen oxidase has been proposed as a cause for

increased coproporphyrin.

**F) Calcium disodium versenate (CaNa2-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 filterpaper 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