MODULE 7.1

Toxicological Chemistry

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Toxic Chemicals and toxicity:

A **toxic chemical** is a substance which is poisoness and which on exposure to above a certain level has a detrimental effect on the human tissue, organs, or biological processes. The term **toxicity** refers to the properties of chemical substances that describe the adverse effects which may be experienced by humans as a result of skin contact, inhalation or ingestion. The effect of a toxicants on the humans can be classified as acute effect and chronic effect. If there is a rapid and serious response to a high but short-lived dose of toxic chemical then it is called acute effect. Acute poisons interfere with essential physiological processes, leading to a variety of symptoms of distress, and even to death if the interference is sufficiently severe. Chronic effects tend to result from low exposure to a toxicant over a relatively long period of time. Chronic effects often set in motion a chain of biochemical events that lead to disease states, including cancer.

Acute toxicity is relatively easy to gauge. The effects of toxins at high-enough levels on bodily function are obvious and fairly consistent across individuals and species. For different chemicals, these levels vary enormously. At some level almost everything is toxic, and the difference between toxic and non toxic is a matter of degree.

The most widely used index of acute toxicity is LD_{50}, the lethal dose for 50 percent of a population. The dose is generally expressed as the weight of the chemical per kilogram of the body weight, of experimental animal on the

assumption that toxicity, scales inversely with the size of the animal. The LD_{50} values are obtained by plotting the number of deaths among the group of experimental animals (usually rats) at various levels of exposure to the chemical and interpolating the resulting dose-response curve to the dose at which half the animals die.

By performing LD_{50} studies for various substances (mass of toxicants per unit body weight) we can assign toxicity ratings to these substances as follows.

(1) Practically nontoxic, >15 g/kg; (2) slightly toxic, 5-15 g/kg; (3) Moderately toxic O.5- 5g/kg; (4) very toxic, 50-500 mg/kg; (5) extremely toxic, 5-50 mg / kg (6) super toxic, < 5 mg /kg.

Kinetic phase and dynamic phase:

The toxicants in the body which are metabolised, transported, and excreted, have adverse biochemical effects. They cause manifestation of poisoning. These processes are conveniently divided into a kinetic phase and a dynamic phase.

Kinetic phase

A toxicant or the metabolic precursor of a toxic substance (**protoxicant**) may undergo absorption, metabolism, temporary storage, distribution, and excretion in the **kinetic phase**. The absorbed toxicant may be passed through a kinetic phase either as an unchanged **active parent compound**, or metabolised to a detoxified metabolite that is excreted, or converted to a toxic **active metabolite.**

Dynamic phase

A toxicant or toxic metabolite **in the dynamic phase** interacts with cells, tissues, or organs in the body to cause some toxic response. The dynamic phase is divided into three major sections and they are primary reaction with a receptor or target organ, a biochemical response and observable effects.

A toxic response may be caused by the reaction of a toxicant or an active metabolite with a receptor . The reaction of benzene epoxide to form an adduct with a nucleic acid unit in DNA resulting in alteration of the DNA is an example. In this case the reaction between a toxicant and a receptor is irreversible.

An example of a **reversible** reaction that can result in a toxic response is illustrated by the binding between carbon monoxide and oxygen-transporting haemoglobin, O_2Hb; in blood (1)

Haemoglobin loses its ability to transfer the oxygen by this reaction.

$$O_2Hb + CO \rightleftarrows CO\,Hb + O_2 \quad \textbf{.....(1)}$$

The kinds of biochemical effects that result when a toxicant is bound to a receptor are the following:(1) By binding to the enzyme, coenzymes, metal activators of enzymes, or enzyme substrates, the enzyme functions are impaired.(2)Cell membrane or carriers in cell membranes are altered.(3)Carbohydrate metabolism is affected. (4) The lipid metabolism is affected resulting in excess lipid accumulation (fatty liver). (5)Interference with respiration, the overall process by which electrons are transferred to molecular oxygen in the biological oxidation of energy- yielding substrates. (6) The protein biosynthesis is either interfered or stopped by the action of toxicants on DNA. (7) The regulatory processes mediated by hormones or enzymes are affected.

Physiological responses to toxicants:

Some prominent chronic responses to toxicant exposure are mutations, cancer, and birth defects and effects on immune system. Gastrointestinal illness, cardiovascular disease, hepatic disease, renal malfunction, neurological symptoms, and skin abnormalities are other observable effects, some of which may occur soon after exposure. Allergy, a kind of condition results when the immune system over reacts to the presence of a foreign agent or its metabolites in a self-destructive manner. Among the foreign substances that can cause such reactions are beryllium, chromium, nickel, formaldehyde, pesticides, resins and plasticizers.

Teratogenesis, mutagenesis and carcinogenesis:-

Teratogenesis:

The chemical species that cause birth defects are called teratogens. These species damage embryonic or fetal cells which result in birth defects. However, mutations in germ cells (egg or sperm cells) may cause birth defects.The biochemical mechanisms include enzyme inhibition by xenobiotics;(synthetic substances that are foreign to living systems), deprivation of essential substrates for fetus such as energy and vitamins and alteration of the permeability of placental membrane .

Mutagenesis:

The essential component of all living things and a basic material in the chromosomes of the cell nucleus is DNA. It contains the genetic code that determines the overall character and appearance of every organism. Each molecule of DNA has the ability to replicate itself exactly, transmitting that genetic information to new cells. But certain chemical reagents, as well as ionising

radiation, are capable of altering DNA. Such changes, or **mutations**, in the genetic code material of an organism can cause cells to misfunction, leading in some cases to cell death, cancer, reproductive failure or abnormal offspring. Hence these substances are of major toxicological concern.

Carcinogenesis :

The role of substances foreign to the body in causing uncontrolled cell replication commonly known as cancer is termed as chemical carcinogenesis. Chemically induced carcinogenesis is thought to involve two distinct stages, referred to as **initiation and promotion**. In the initiation stage chemical carcinogens alter the DNA in a manner such that cells replicate uncontrollably and forms cancerous tissue. In the second or promotion, stage of development, affected cells no longer recognise growth constraints and tumor develops. Promoters can increase the incidence rate of tumors among cells that have already undergone initiation, or they can shorten the latency period between initiation and the full carcinogenic response. The model of initiation followed by promotion suggests that some carcinogens may be initiators, others may be promoters, and some may be complete carcinogens capable of causing both stages to occur.

One example of a chemical that has definitely been established as human carcinogen is vinyl chloride, CH_2=CHCl which is known to have caused a rare form of liver cancer.

Neurotoxins:

These are metabolic poisons which attack nerve cells (neurons) which regulate body activities. Pb,Hg kill nerve cells and cause permanent neurological

damage. Ether, chloroform, anesthetics, DDT and aldrin disrupt nerve cell membrane, necessary for nerve action.

Organophosphates (malathion etc) and carbamates inhibit acetylcholinesterase, an enzyme that regulate signal transmission between nerve cells and the tissues or organ they innervate. The mechanism of inhibition by these insecticides is discussed in detail in the next chapter 4.2.

Toxicity Of Metals, Inorganic Compounds & Organic Compounds:

There are a number of chemicals including a large number of metals and metalloids in the environment. Some of these are toxic and others non-toxic. Due to industrial discharge, these elements find their way in air, water and soil in our environment. They get into our biological system through food chain and disturb the biochemical processes , leading in some cases to fatal results. The biochemical effects of some of the metals, metalloids, inorganic compounds and organic compounds will be discussed.

Toxic elements:

The biosphere has evolved in close association with all the elements of the periodic table and organisms harnessed the chemistry of many metal ions for essential biochemical functions. These elements are required for viability, although in small doses. When the supply of that essential element is insufficient, it limits the viability of the organism, but when it is present in excess, it exerts toxic effects. Thus there are optimal dose for all essential elements. This optimum varies widely for elements since chemistry of the element varies. For example comparing Cu^{2+} with Fe^{2+} or Fe^{3+}, the former binds strongly to nitrogenous bases, including histidine side chain of proteins whereas neither Fe^{2+}

nor Fe^{3+} strongly bind to nitrogenous bases. Therefore Cu^{2+} is more likely to interfere than iron with critical sites in proteins. At higher levels iron is harmful, partly because it can catalyse the production of oxygen radicals, and partly because excess iron can stimulate the growth of bacteria and aggrevate infections. Cr(III) is considered as essential element but Cr(VI) is carcinogenic.

Cadmium along with lead, mercury and arsenic is a soft Lewis acid, with particular affinity for soft Lewis bases, such as the sulphydryl side chain of cysteine amino acids. Thus it is possible that the heavy metals exert their toxic effects by bonding with critical cystein residues in proteins; although the actual physiological consequences vary from one metal to another.

Mercury

Mercury enters the environment mainly through industrial discharges such as from chloroalkali plants, electrical apparatus production plants, agricultural industry which use a large number of fungicide for seed dressings. Sewage effluent from these industries sometimes contain mercury upto 10 times the concentration of natural water. When the mercury is adsorbed on sediments in the water bodies, the sulphate reducing bacteria in sediments generate methyl mercury [CH_3Hg^+ and $(CH_3)_2Hg$; $(CH_3)_2Hg$ Volatilises out) and release to the waters above, where it is absorbed by the fish from the water. The CH_3Hg^+ ion forms CH_3Hg Cl in the saline biological fluids, and this neutral complex passes through biological membranes and is distributed throughout the tissues of the fish. In the tissues the chloride is displaced by peptide sulphydryl groups, since mercury has an affinity for sulphur ligands, the methyl mercury is eliminated only slowly and is therefore subject to bioaccumulation, when little fish is eaten by

bigger fish. Biomethylation of mercury occurs in all sediments and fish everywhere have some mercury.

But the levels are greatly elevated in bodies of water for which sediments are contaminated by mercury from waste effluents. The worst case of environmental mercury poisoning occured in the 1950s in Minimata, Japan, where fish accumulated methyl mercury to levels approaching 100 ppm. Thousands of people were poisoned and hundreds died from the poisoned fish. Since methyl mercury is able to cross the blood-brain barrier, the affected people suffered all symptoms of brain dysfunction. Similarly methyl mercury can pass from mother to fetus, and a number of minimata infants suffered mental retardation and motor disturbance before the cause of poisoning was identified.

Elemental mercury enters the body through inhalation and carried by the blood stream to the brain, where it penetrates the blood brain barrier and again all symptoms of brain disfunction will occur. Hg_2^{2+} is not toxic since it forms insoluble chloride in the stomach. Hg^{2+} again is poisoness and forms strong complexes with sulphur containing amino acids and proteins. This ion however does not get access to biological cell.

Cadmium:

The chemical properties of cadmium are much closer to zinc than mercury. The main sources for cadmium in the environment are from coal, zinc mining, refining of metals and tobacco smoking. The cadmium build in agricultural soils is of concern. These cadmium inputs to soils are mainly from air borne deposition from commercial phosphate fertilisers, which contain cadmium as a natural constituent of phosphate ore. The cadmium concentration would

further increase with the use of fertiliser from sewage sludge (which is often contaminated with cadmium and other metals).

Soil conditions were certainly a factor in the only known case of wide spread environmental cadmium poisoning, which occured in the Jinzu valley of Japan. Irrigation water drawn from a river that was contaminated by zinc mining and smelting complex led to high levels of cadmium in rice. Hundreds of people in the area developed degenerative bone disease called *itai-itai* due to interference of cadmium with Ca^{2+} deposition. Their bones became porous and subject to collapse. Chronic exposure to cadmium has been linked to heart and lung disease, immune suppression, and liver and kidney disease. Cadmium attacks the active sites of enzyme inhibiting essential function. The enzyme inhibited by Cd^{2+} include adenosine triphosphate, alcohol dehydrogenase, anylase, carbonic anhydrase, peptidase activity in carboxy peptidase and glutamic oxaloacetic transminase. As mentioned the Cd^{2+} requestering protein metallothionen provides protection until its capacity is exceeded. Since metallothionen is concentrated in the kidney, this organ is damaged first by excessive cadmium. The rest of the cadmium is stored in the body and accumulates with age. When excessive amounts of Cd^{2+} is ingested, it replaces Zn^{2+} at key enzymatic sites causing metabolic disorders.

Arsenic:

Arsenic occurs in water as a result of mineral dissolution, industrial discharges or the applications of insecticides. As (III) is more toxic than As(V) , because it binds more readily to sulphydryl groups of enzyme and inhibits the enzyme action. The inhibitory action is based on the inactivation of pyruvate dehydrogenase through its complexation whereby the generation of adenosine

triphosphate (ATP) is prevented. By virtue of its similarity with phosphorous, arsenic (III) interferes with some biochemical processes involving phosphorous. In the ATP generation, the enzymatic synthesis of 1,3-diphosphoglycerate from glyceraldehyde3-phosphate is an important step.As (III) interferes by producing 1-arseno-3-phosphoglycerate instead of 1,3 diphosphoglycerate thereby inhibiting the metabolic processes. It acts to coagulate proteins and forms complexes with coenzymes. Like mercury, arsenic may be converted to more mobile and toxic methyl derivatives such as methylarsenic acid and dimethyl arsenic acid. In another recent discovery, low levels of arsenic were found to inhibit activation receptors that turn of many genes that suppress cancer and regulate blood sugar and at high levels it is known to trigger diabetes as well as cancer.

Arsenic in drinking water is a slow poison. The first symptoms are discolourisation of the skin. Later these develop into cancers and the liver and kidneys also deteriorate.

Lead

The main sources for lead in the environment are from auto exhaust from gasoline, paints storage batteries and pipes. Lead can contaminate water either from lead-based solder used in pipe and fitting connections. In contact with O_2 -bearing water, the metallic lead can be oxidised and solubilised.

$$2Pb + O_2 + 4H^+ \rightleftharpoons 2Pb^{2+} + 2H_2O \quad \textbf{\textit{.....(1)}}$$

The dissolution rate is strongly pH dependent. The solubility of lead in soft water is higher when compared to hard water which has a high pH. Carbonates precipitate lead as sparingly soluble $PbCO_3$. Another major source of lead

exposure is leaded gasoline in which lead tetraethyl is added to improve octane rating by scavenging radicals and inhibiting pre-ignition. These toxic compounds are readily absorbed through the skin and in the liver and they are converted to trialkyl-lead ions, R_3Pb^+, which like methyl mercury ions are neurotoxins.

The fine particles emitted by automobiles are retained within the lungs and are absorbed by the body with an efficiency of about forty percent. These particles can travel far on air currents. However most of the particles settle out not far from where they are generated, contaminating the dust near road ways and in urban areas with lead.

Lead enters the blood stream once absorbed in the body, and moves from there to soft tissues. Lead is deposited in bone, because Pb^{2+} and Ca^{2+} have similar ionic radii. The body maintains about 15-25 µg of lead per 100g of whole blood. The body responds to any increase in lead intake by excreting in the urine as much as possible and the remainder is stored primarily in the bones. At elevated blood lead levels it inhibits the enzymes involved in the biosynthesis of heme, the iron-porphyrin complex that binds to haemoglobin and serves as a binding site for O_2.

The biochemical mechanism for lead's effects on nerve cells is uncertain, but the diminution of nerve conduction velocity can be detected at low blood lead levels; higher levels cause nerve degradation. Studies have shown that lead even at very low levels (as low as 5mg/dL) was found to cause impairment in growth, hearing and mental development of children.

The molecular mechanisms of lead toxicity has not been exactly identified. It probably involves leads's ability to bind to nitrogen and sulphur ligands, thereby

interfering with the function of critical proteins like ferrochelatase. By intravenous injection of chelating agents, lead can be cleared from the body. The chelators compete for the protein bind sites, and resulting Pb^{2+}-chelate complexes are excreted by the kidneys. The chelating agents are administered as Ca^{2+} complex, to avoid stripping of calcium or other weakly bound metals from the body. The strongly binding Pb^{2+} displaces the Ca^{2+} and is removed selectively.

Selenium:

Selenium is not widely used in industry. Its major use is in the manufacture of electrical components: photoelectric cells and rectifiers. It is an essential element at low levels but toxic at higher concentrations . It is comparable to arsenic in toxicity towards man and animal, giving rise to similar symptoms. It has been suspected of being a carcinogenic agent. The element itself and the heavy metal sediments are insoluble. Elemental selenium is not rapidly oxidised and is slowly available to plants from soils. Selenous acid, $H_2SeO_3^-$ is very mobile in aqueous environment and is readily available to plants, SeO_4^{2-} is fairly strong oxidising agent and readily reducible to SeO_3^{2-} under most environmental conditions.

Beryllium:

The main sources for beryllium in the environment are from coal combustion, nuclear power and space industries. Most beryllium emissions are in the form of metallic powder or beryllium oxide particulates. Acute beryllium exposure is a very serious occupational problem affecting the mucus membranes of the eyes and lungs. As low as 0.01 to 0.1 µg m^{-3} concentrations of beryllium leads to a chronic condition known as berylliosis. It is a systemic poisoning

starting with progressive shortness of breadth, weight loss and cough, and finally affects many organs including the heart. It is a suspected carcinogen.

Toxicity Of Some Inorganic Compounds:

Cyanide: Cyanide gains access to the water environment through the discharge of rinse waters from plating operations and refinery and coalcoking waste waters. Both hydrogen cyanide and cyanide salts are rapidly acting poisons and even a dose of 60 to 90 mg is sufficient to kill a human. Cyanide exerts its toxic action by inhibiting oxidative enzymes from mediating the process by which O_2 is utilised to complete the production of ATP in the mitochondria. Metabolically, cyanide binds to iron (III) in ferritochrome oxidase enzymes, thus preventing its reduction to iron (II) in the oxidative phosphorylation process in which O_2 is utilised. The crucial enzyme is inhibited since, ferrous cytochrome oxidase is not formed which is required to react with O_2. Thus utilisation of oxygen in the cells is prevented and the metabolic processes cease.

Carbonmonoxide: The significant source for CO in the environment is from transportation. Carbonmonoxide when inhaled, passes through the lungs and diffuses directly into the blood stream where it combines with haemoglobin to form carboxy haemoglobin (COHb).

$$O_2Hb + CO \rightarrow COHb + O_2 \quad \textbf{.....(2)}$$

The affinity of CO for haemoglobin is 210 times greater than that of oxygen. As a result the amount of haemoglobin available for carrying oxygen for body tissue is considerably reduced. The body tissues are thus deprived of their oxygen supply and death could result by lack of oxygen. In addition, the

presence of COHb in the blood retards the dissociation of remaining oxyhaemoglobin, so the tissues are further deprived of O_2.

Carbon monoxide is the common cause of accidental poisoning. The toxic effects of CO of various concentrations is shown in table 1.

Table 1 Co concentration in air and toxic effects

CO Concentration in air ppm	Toxic effects
10	impairment of judgement
100	dizziness,headache
250	loss of consciousness
1000	rapid death

Oxides of nitrogen:

Nitrous oxide (N_2O) is used as an oxidant gas and in dental surgery as a general anesthetic. It is a central nervous system depressant and can act as asphyxiant.Nitric oxide (NO) and nitrogen dioxide ($NO_{2)}$ are the two major oxides of nitrogen which affect human health. NO, as it is does not show any adverse health effects. But it becomes toxic when it is oxidised to NO_2.

NO_2, after inhalation reaches the moisture filled alveoli of the lungs. There it is converted into nitrous acid and nitric acid which are highly irritating and cause damage to the lung tissues. Biochemically NO_2 disrupts lactic dehydrogenase and some other enzyme systems. Free radicals particularly HO• are likely formed in the body by the action of NO_2 and the compound probably causes lipid peroxidation in which the C=C double bonds in unsaturated body lipids are attacked by free radicals and undergo chain reaction in the presence of

O_2 resulting in their oxidative destruction. NO_2 in combination with hydrocarbons acts as the initiator of photochemical smog leading to the production of secondary pollutants like the oxidants. These oxidants are the ones that cause damage to human health.

Sulphur dioxide:

The main concern of SO_2 in urban atmospheres arise not from SO_2, but from the changes it undergoes in the atmosphere such as the formation of H_2SO_4 and sulphate aerosols. The sulphate particles can be carried deep into the lungs, causing even more severe health problems. SO_2 can also be absorbed on small particulates such as the salts of iron, manganese and vanadium present in the atmosphere and thus enter the alveoli. There in the presence of moist air, SO_2 is oxidised to H_2SO_4 and the particulates act as catalysts in enhancing the oxidation process.

Ozone:

Ozone is a very reactive substance. It causes the cracking of synthetic rubbers at atmospheric levels of 0.01 to 0.02 ppm. It also attacks fabric fibres and adverse effect increase in the order: fibres made of cotton, acetate, nylon, and polyester. The fading of fibres and the cracking of rubber are attributed to ozone's oxidising ability.

Ozone has several toxic effects. Inhalation of ozone at 1 ppm level causes severe irritation and headache and sometimes causes severe pulmonary edema. Ozone generates free radicals in the tissue. These reactive species are the causes for lipid peroxidation, oxidation of sulphydryl groups, and other destructive oxidation processes.

Asbestos

"Asbestos" is an industrial term for a number of hydrated silicates with an approximate formula $Mg_3\ P(Si_2O_5)\ (OH)_4$. They separate into strong flexible fibres upon crushing and processing. Inhalation of asbestos dust or fibres can cause a disabling lung disease known as asbestosis. The disease is characterised by shortness of breadth and pleural calcification. Asbestos has also been proved to induce lung cancer. The fibres line the membranes of the lungs and abdomen and this can lead to mesothelioma, an incurable and fatal cancer.