Toxic Effects of Xenobiotics

The exposure of animals to certain doses/concentrations of toxicants brings about some alterations in the organisms. Such alterations are termed as *responses* of organisms or *effects* of toxicants. When the criterion of effect is death of organisms, the obtained response is *'all or none'* type. Following exposure to a toxicant, the animal either dies or survives, *i.e.* either the animal responds fully to the toxicant and the response is death of the animal or it does not respond at all. Hence, this type of effect is termed as *'all or none'* type. The toxicant induced effects in organisms can be grouped as below:

(a) Local and Systemic effects *Local effects may be described as injuries caused by chemicals in biological system at the site of first contact.* The local effects may be produced by caustic substances on gastrointestinal tract upon oral ingestion, by corrosive substances on the skin or by irritant gases and vapours on the respiratory tract upon inhalation.

Besides, *certain other substances produce harmful effects when they are absorbed and distributed from the point of their entry to a distant site, i.e*. the site of their action. This type of effect is termed as *systemic effect*. For example, organochlorine, organophosphorus and carbamate pesticides applied on the body of organisms reach the central nervous system after crossing various barriers and then produce toxic effects by inducing certain alterations in the normal functioning of the system. The systemic poisons do not cause similar degree of effects to all organs. They elicit major effects to one or two organs (or tissues) only. Such organs or tissues are designated as *target organs* or *target tissues.* The target organ does not always possess the highest concentration of the chemical. For example, a high amount of DDT is accumulated in adipose tissue, but it produces its effect on the central nervous system.

Still other substances produce both types of effects. For example, tetraethyl lead produces effects on skin at the site of application and is then transported systemically to produce typical effects on central nervous system and other organs.

(b) Immediate and Delayed effects Certain toxicants produce immediate toxic effects. Such effects develop rapidly after a single administration of the substance. An extreme example of immediate effect is that produced by cyanides, especially by potassium cyanide (KCN).

Several other chemicals produce effects after a lapse of some time. This type of effect is termed as *delayed effects*. Chemicals induce carcinogenic effects after a latent period of 20-30 years in human beings. This is an extreme example of delayed effects. Some organophosphorus anticholinesterase agents also cause delayed neurotoxicity.

(c) ***Reversible and irreversible effects*** Some chemicals produce adverse effects that disappear following withdrawal of exposure. This is known as *reversible effect*. Certain other alterations produced by other toxicants in the biological system do not disappear even after considerable time following withdrawal of the exposure. This type of effect either persists or progresses even when exposure is withdrawn and is termed as *irreversible effect.* Generally, the pharmacological effects are reversible, such as those toxic effects involving the inhibition of an enzyme whereas direct tissue damage (histopathological effects) is often irreversible. The

inhibition of cholinesterase activity following exposure of organisms to organophosphorus and carbamate pesticides is irreversible whereas that inhibited by antimetabolites is reversible. In case of the latter, after the withdrawal of exposure, the activity of enzyme is recovered.

Various other chemicals cause pathological injury to certain tissues of the animals. The ability of tissue to regenerate determines whether the effect is reversible or irreversible. Damage to highly regenerative tissue, like liver is reversible whereas injuries to central nervous system are irreversible. Carcinogenic effects of toxicants are also irreversible toxic effects.

(B) Basis of toxic effects

The toxicants attack at different vulnerable sites in the biological systems to induce the toxic effects. Thus, there can be several bases for inducing toxic effects in the biological systems.

(1) Toxic effects through interference in the activity of enzymes

Certain enzymes or enzyme systems are always involved in the initiation and catalysis of biochemical reactions in the biological systems and these reactions form the basis of functioning of the biological systems. The interaction of a toxicant with certain enzyme impairs the particular function of organism with which the enzyme is associated. Consequently, the biochemical pathways are blocked and toxic effects are manifested. Toxicant induced enzymatic changes may be grouped into the following categories:

(i) Enzyme inhibition The toxicants bind with the active sites of enzymes and thereby inhibit the rate of enzyme catalyzed reactions. This process is often termed as ***enzyme inhibition.*** Usually, two types of enzyme inhibition take place in the biological system.

(a) When the binding of toxicant is for short duration and thereafter the enzyme activity is recovered; the inhibition is termed as *reversible inhibition*, and

(b) When the binding is for long duration because of covalent or other stable bonding between toxicant and target enzyme, the inhibition is termed as *irreversible inhibition.*

The organophosphorus pesticides bind with strong covalent bonds at the active site of acetylcholinesterase (AChE) and thereby alter the physiology of the nervous system by blocking the transmission of impulses at the synapses. Thus, these toxicants irreversibly inhibit AChE activity. Carbamate pesticides also inhibit the activity of this enzyme, but the rate of hydrolysis of enzyme-pesticide complex is relatively faster. Hence, carbamates are believed to cause reversible inhibition of AChE.

Certain organochlorinated pesticides (such as, DDT) are believed to inhibit the activity of Na^+-K^+ATPase and thereby affect the permeability of Na^+ and K^+ ions across the nerve membranes and thus affect the conduction of impulses across the nerve fibres.

Certain heavy metals, *viz.* mercury, lead, arsenic, *etc.* interact with sulphydryl group (-SH) of a large number of enzymes and thus inhibit the activity of a large number of enzymes. Formation of covalent bond between toxicant and sulphydryl group of enzyme inactivates the enzyme concerned, as the active site of enzyme is blocked by the toxicant and thus not available for action with its normal substrate.

An enzyme, α-amino levulinic acid dehydratase (ALAD) catalyses the condensation of two molecules of amino levulinic acid to porphobilinogen and is thus involved in porphyrin and RBC metabolism. Lead inhibits the activity of this enzyme and thus interferes with RBC metabolism. Other toxicants, such as EDTA, mercury, copper, silver, manganese also inhibit the activity of this enzyme, but the inhibition in ALAD activity appears to be relatively lead specific.

Certain antimetabolites are structurally similar to normal substrates of enzymes. They bind with active sites of such enzymes and thus render the enzymes unable to react with their natural substrates. This type of enzyme inhibition is reversible inhibition. An example of this type of inhibitor is folic acid antagonist, ***methotrexate,*** which is used as a cytostatic in the treatment of cancer. Methotrexate inhibits enzyme systems that are essential for the synthesis of amino acids, and purine and pyrimdine derivatives. Amino acids are essential for protein synthesis and purine and pyrimdine derivatives for nucleic acids formation. Alteration in protein and nucleic acid metabolisms inhibits the cell proliferation.

(ii) Uncoupling of biochemical reactions During biological oxidations some energy is released which is stored in high energy phosphate molecules, like ATP. This energy is later utilized in certain biochemical processes, such as synthetic reactions and chemo-mechanical processes of muscle contraction. The uncoupling agents, such as dinitrophenol and dinitro-ortho cresol, interfere with the synthesis of energy rich phosphates which results in the liberation of energy as heat rather than its storage; ultimately resulting in the fever, *i.e.* rise in body temperature.

(iii) Inhibition of photosynthesis of plants During photosynthesis radiation energy is trapped by the green parts (*i.e.* chlorophyll granules) of plants. The entrapped energy is utilized to split the water molecule into oxygen and hydrogen. Usually, hydrogen is transferred to NADP to form NADPH by a redox system. This hydrogen is later utilized to form glucose from CO_2 with the help of certain enzymes. Certain herbicides, *viz*. monuran, interfere with the first step of photosynthesis, *i.e.* the photolysis of water. Another group of herbicides, *viz.* paraquat and diquat, interfere with the transference of hydrogen to NADP.

(iv) Synthesis of lethal intermediates Certain toxicants/antimetabolites that are structurally similar to natural substrates of enzymes are incorporated into the biochemical chain of reactions. They are ultimately converted into toxic end products, which are strong inhibitors of certain enzymes/enzyme systems. Thus, they may block the biochemical pathways. For example, *fluoroacetate* is converted into *fluorocitrate* in the body of organisms by certain enzyme and the latter is a strong inhibitor of an enzyme, *aconitase*. This enzyme converts citric acid into isocitric acid. Owing to the inhibition of aconitase by fluorocitrate, citric acid cycle is blocked.

(v) Removal of metallic ions from metalloenzymes Certain metal ions, such as iron, copper, cobalt, zinc, manganese, *etc.*, act as cofactors and are essential components of certain enzymes (*i.e.* metalloenzymes) for their normal activity. Some chelating agents, like dimercaprol, dithiocarbamates and hydroxy quinone derivatives, are capable of withdrawing these essential metal ions from enzymes/ enzyme systems. For example, dithiocarbamates withdraw copper ions from an enzyme, *acetyl dehydrogenase,* which catalyses the degradation of alcohol, thus inactivates it ultimately blocking the degradation of alcohol. Iron containing *cytochrome oxidase system* plays an important role in electron transport chain. Hydrogen cyanide(HCN) has capacity to withdraw iron from this enzyme system and thus may disrupt the entire process of aerobic respiration.

(2)Toxic effects through blockade of oxygen transport

Certain toxicants are capable of blocking oxygen transport in higher organisms either by damaging RBCs or by reacting with haemoglobin molecules that impairs oxygen carrying capacity of the blood. This may lead to reduction in O_2 content at cellular level (*cellular anoxia*), which may cause severe damage to the biological system.

(i) Carbon monoxide poisoning Carbon monoxide is an important atmospheric pollutant. It produces difficulty in breathing, causes headache and results in irritation of mucous membranes. It combines with respiratory pigment (*e.g.* haemoglobin) of blood and decreases its oxygen carrying capacity and thus produces *anaemic hypoxia*. Exposure to very high concentration of carbon monoxide can result in sufficient haemoglobin saturation to cause death in minutes.

(ii) Methemoglobin formation Methemoglobin is oxidized form of haemoglobin with no oxygen carrying capacity. Many aromatic amines and nitro compounds undergo reduction in higher organisms that accelerate the formation of methemoglobin. Erythrocytes have got the ability to convert back methemoglobin to haemoglobin. But, they fail to keep pace if the rate of methemoglobin formation is faster. Therefore, O_2 transport capacity of the blood is drastically impaired.

(iii) Sulphaemoglobin formation Sulphur containing compounds, like sulphonamide, give rise to sulphaemoglobin under the influence of substances, which give rise to methemoglobin. Sulphaemoglobin is unable to transport O_2 and its formation inhibits O_2 transport in living-beings.

(iv) Haemolysis Some toxicants, such as surfactants and hydrazine derivatives, are capable of rupturing RBC membranes resulting in discharge of haemoglobin in the blood stream. Consequently, there is drastic reduction in O_2 carrying capacity of the blood. This may lead to rise in bilirubin content of the blood and symptoms of jaundice and anaemia may appear.

(3) Toxic effects through interference in the synthesis and functions of nucleic acids and proteins

DNA, RNA and proteins collectively constitute the most important functional components of the cell and regulate its activity, growth, division and ensure same genetic make up in the daughter cells. Toxicants induced alterations in synthesis and functioning of these macromolecules may bring about carcinogenicity, mutagenicity and teratogenicity.

(i) Interference in duplication of DNA Chemicals of actinomycin group and pentavalent alkylating substances are capable of binding with DNA chain and thus make its duplication impossible. Alkylating agents may form chemical bridges between two DNA strands and disturb its replication. Consequently, during cell division chromosomes adhere together and undergo fragmentation.

(ii) Interference in RNA synthesis An enzyme, ***RNA polymerase***, plays an essential role in RNA synthesis. Certain chemicals (*e.g.* Refampicin) inhibit this enzyme and thus disturb the process of transcription of genetic information from DNA to RNA.

(iii) Interference in protein synthesis Certain antibiotics, such as pauromycin, streptomycin and gentamycin, inhibit protein synthesis of bacteria by masking the translation of information present on messenger RNA.

Pauromycin is structurally similar to tyrosine-t-RNA. In the presence of the former, the latter is unfit for incorporation in amino acid chain; its substitution results in the formation of short chains of amino acids and thus the protein synthesis is inhibited.

(iv) Interference in the synthesis of various components of nucleic acids and protein molecules In addition to the systems of DNA, RNA and protein synthesis, cells also possess systems for the synthesis of various components of protein and nucleic acid chains. Interference in the activity of these enzyme systems results in shortage of basic ingredients of these macromolecules.

(v) Interference in the regulatory processes that determine the activity pattern of a cell Regulatory processes exist in the cell, which determine when a protein shall be formed? And how much of it shall be produced? Similarly, the portion of DNA chain carrying information for undesired protein is screened off, probably by the basic proteins and the system of derepression exists within the cell, which triggers the formation of m-RNA for proteins required by the cell. Certain substances or their biotransformation products may cause repression of a particular portion of DNA chain, which is responsible for the formation of m-RNA; thereby inhibit the formation of required protein. Thus, the formation of protein molecule may be hindered by repression of DNA molecule; due to which the required m-RNA templates are not available.

Some of the important consequences of interference of toxicants in the synthesis and functioning of DNA, RNA and proteins are being discussed below:

(a) Cytostatic action Cytostatic action is an inhibition of cell division and therefore of tissue growth, which is based on cell proliferation. Interference in duplication of DNA, transcription and translation of genetic information, protein synthesis and synthesis of any of the components of DNA, RNA and protein molecules, besides interference in the regulatory mechanism determining the activity pattern of a cell may cause cytostatic action. Certain biological alkylating agents having cytostatic action are used in cancer chemotherapy. But, these substances have very poor selectivity in their action. Therefore, besides inhibiting the growth of malignant tissues, they often also check the growth of some other useful and highly proliferating tissues, such as bone marrow and intestinal mucosa.

(b) Immunosuppressive action Immunosuppressive action is related to cytostatic action of chemicals. They cause suppression of immunological defence mechanisms by suppressing the proliferation of certain cells, especially the lymphocytes. Being poorly selective in action cytostatic substances also suppress the immune system of an organism.

(c) Teratogenic action The nature has provided a barrier, *i.e.* placental barrier, for the protection of developing embryos, especially in higher mammals. Certain lipophilic substances can cross this barrier and affect the growth and development of embryos, ultimately leading to their abnormal development. This type of action of toxicants is termed as *teratogenic action*. Interference in rapidly duplicating DNA molecules and active cell divisions may cause all types of such abnormalities.

(d) Carcinogenic action Changes in the properties of DNA molecules may cause disordered growth of cells, which can invade and destroy other tissues. In fact, carcinogenic action involves development of tumours, which are abnormal, functionless and non-inflammatory mass of cells formed from existing tissues. Various toxicants are known that can cause disturbances in the process of DNA duplication and thus, can introduce abnormalities in this vital molecule.

(e) Mutagenic action Some alkylating agents induce chemical changes in DNA molecule that may alter its properties and thus, the genetic properties of daughter cells. Such processes are

termed as *genetic mutations* and the substances causing them as *mutagens*. The effects of mutagens are often seen only after a few generations, because the mutations are usually recessive and become manifested only when two individuals with mutations on the same point of DNA molecule cross breed. The period of latency for the effect of mutagenic substances is usually very long. Inbreeding will markedly shorten the latency period.

(4) Hypersensitivity and Allergy

The term hypersensitivity is used to describe reactions that cause development of increased sensitivity of a biological system to various substances. These reactions involve the formation of specific antibodies for the foreign substances.

In hypersensitivity reactions, the foreign substances (*i.e.* allergens) do not act as antigens themselves. But, the substances (or their biotransformation products) induce the formation of antigens by reacting with protein molecules that finally lead to the development of antibodies. If allergen is a polypeptide, it can directly induce the formation of antibodies and in such cases, it is identical with that of antigen.

The basis of violent hypersensitivity reaction lies in the fact that once antibodies are developed in living organisms, any subsequent exposure to the same or similar chemical agents causes antigen-antibody reactions. During which substances, like histamine and bradykinin, are liberated. In fact, these substances are responsible for the toxic responses. The degree of hypersensitivity reaction is dependent on the concentration of antibodies formed and the strength of exposure to foreign chemical.

Usually, allergic reactions are exhibited on the skin and respiratory tract of an organism. Practically, any substance can cause allergic reactions under certain set of conditions that depend on the constitution of the individual and properties of chemical agent. Some compounds, like dinitrochlorobenzene and quinine, are capable of generating hypersensitivity reactions in most of individuals.

(5) Direct chemical irritation of tissues

Skin is a protective layer against various xenobiotic substances. Chemically reactive toxic agents can cause direct damage to this layer, when they come in contact with it. Various keratolytic agents and blister forming chemicals may cause direct skin injury. Strong alkaline solutions, such as those of NaOH, KOH and strong acids, such as sulphuric acid, hydrochloric acid, nitric acid, *etc.*, are capable of causing much damage. Drastic changes in pH caused by these chemicals bring about changes in keratin of skin that may at times induce rapid absorption of water from the surrounding tissues and result in swellings. Concentrated nitric acid not only changes the pH, but also results in nitrification and oxidation of various constituents of skin that ultimately turns yellow.

Organic solvents dissolve away the protective lipid layer and this makes the skin more pervious of other chemical agents, which enter through pores or along the root of hairs; causing skin irritation, dermatitis, *etc.*

Chlorine gas, phosgene and nitrogen mustard gas are strong skin irritants. These cause oedema in lungs. Nitrogen mustard gas also causes blister formation on the skin. Gases, like NO_2, O_3 and SO_2, cause irritation in nasal mucosa membranes and membranes of deeper parts of respiratory tract. Very low concentration of tear gases cause extreme irritation of conjunctiva and

stinging pain in the eyes, followed by copious lacrimations. Large quantities of this gas may affect mucous membrane and cause pulmonary oedema.