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MEV-004
Environmental Toxicology

INTRODUCTION TO TOXICOLOGY

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Indira Gandhi National Open University
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Block

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INTRODUCTION TO TOXICOLOGY

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February, 2019

ISBN: 987-88-88498-87-6

Further information on the IGNOU courses may be obtained from the University's office at Maidan Garhi, New Delhi or the official website of IGNOU at www.ignou.ac.in

Printed and published on behalf of IGNOU, New Delhi by Registrar, MPDD, IGNOU, New Delhi.

Laser Typeset by Tessa Media & Computers, C-206, A.F.E.-II, Okhla, New Delhi.

Printed at:

COURSE INTRODUCTION

Welcome to the Post Graduate Diploma in Environmental and Occupational Health (PGDEOH) of IGNOU. The curriculum prepared for this programme is relevant and significant in the present day scenario. This programme is in consensus with the mission of Environmental and Occupational Health which is to prevent adverse health effects related to chemical and biological contaminants in the Environmental and Occupational exposures through education, research and service.

This core course is entitled “Environmental Toxicology”. This course consists of 4 blocks comprising of 15 Units.

Block 1 deals with the introduction to the field of toxicology. The block deals with the basic concepts of toxicology, types of toxicants and their effects on human health. The importance of toxicity assessment and types of tests for assessment is also described in detail. The block also describes how toxic substances can enter the human body and cause deleterious effects. The different types of toxicants namely chemical, biological toxicants and their effects have also been described. Finally the block explains the toxicity assessment and the types of tests that can be used for assessment of toxicity.

Block 2 deals with the basics of eco-toxicology and concepts related to immunotoxicology, neurotoxicity and reproductive toxicity. The block also describes toxic biotransformations and the various effects of toxicants on the environment and human health. The concepts of detoxification and mechanisms have been described with suitable examples.

Block 3 deals with environmental toxicity risk assessment. The block vividly describes the acceptable limits of toxicants and how estimating health risks are most essential. Finally the block focuses on toxic remediation of the air, water and soil environments with suitable case studies.

Block 4 deals with environmental cytotoxicity and genotoxicity. The terms such as mutagenecity, carcinogenecity and teratogenecity has been explained with the mechanisms of action. The block summarizes with the prevention of cancers and mutagens and explains the screening methods for the same.

All these Blocks will provide you with sufficient knowledge about the toxicants, their mode of action and how toxicity risk assessments can be done.

INTRODUCTION TO BLOCK 1

This block focuses on the concepts of toxicology, various toxicants found in the environment and their effects on the environment and also on human health. The quality of our environment is degrading and we are exposed to a variety of pollutants in everyday life. These pollutants can have acute, chronic, sub-acute and toxic effects on the living organisms as well as human beings. Even the substances such as paints, detergents, cleaning solutions, cosmetics that we use can have hazardous chemicals giving rise to toxic effects on our health. The different types of toxicants namely chemical, biological toxicants and their effects have also been described. The block finally describes the different tests used in toxicity assessment.

Unit 1 deals with the definition of toxicants, the various sources of toxicants and the mode of entry of toxic substances along with their action. The routes of absorption of toxic substances such as skin, lung, gastrointestinal tracts; and the distribution and storage of toxins in human tissues such as the plasma, kidney, fat, bone, blood and the placenta is also described in detail.

Unit 2 deals with the different types of chemical toxicants and their effects on the environment and human health. Chemical toxicants are released into the environment in different ways, and they can be transported through several pathways. The unit discusses that the chemical toxicants released into the atmosphere exert adverse effects on humans and other terrestrial and aquatic organisms through ingestion, occupational exposure, environmental exposure, as well as accidental and intentional poisoning.

Unit 3 deals with biological toxicants and their effects of human health and environment. Biological toxicants are chemical substances which are toxins produced by living organisms. The unit details on food intoxication from members in the animal and plant kingdom and how we can protect ourselves from these pathogens.

Unit 4 deals with the toxicity assessment and the types and classification of toxicity tests including exposure assessments. In addition to dose, other factors may also influence the toxicity of the compound such as the route of entry, duration and frequency of exposure, variations between different species and variations among members of the same species. The unit finally discusses the potential hazards to humans as acute, subchronic and chronic toxicity.

UNIT 1 INTRODUCTION TO TOXICANTS

Structure

1.0 INTRODUCTION

We are exposed to different substances in our everyday life and some of these may be toxic to our health. The quality of our environment is also decreasing and people are exposed to a variety of pollutants. These pollutants can have acute, chronic, sub-acute and toxic effects on the living organisms as well as human beings. Substances that we use in daily life starting from paints, detergents, cleaning solutions, cosmetics etc. can have hazardous chemicals which can have toxic effects on our health. Rachel Carson is considered the mother of environmental toxicology. She published the book Silent Spring in 1962 which discussed the toxic effects of the pesticide DDT. Living organisms can be exposed to toxic substances at any stage of their life cycle. They can be accumulated in the fatty tissues and lead to bioaccumulation. This can lead to biomagnifications of specific toxicants. In this unit let us learn about toxins, toxicants, their types, sources and the mechanisms of their action.

1.1 OBJECTIVE

After reading this unit, you should be able to:

- define toxins and toxicants;
- understand the different concepts and terminologies used in environmental toxicology;
- explain the different sources of toxicants;
- describe the routes of exposure to toxicants;
- understand the mechanisms and site of toxic action by toxicants; and
- explain how toxins are stored in the various tissues of the human body.

1.2 DEFINITIONS AND CONCEPTS

1.2.1 Definitions

Let us now learn about some definitions and terms commonly used in environmental toxicology.

a) *Environmental toxicology*: It is a branch of science that deals with the harmful effects of different physical, chemical and biological agents on living organisms. It is multidisciplinary in nature.

b) *Ecotoxicology:* This is a sub-discipline of environmental toxicology. This deals with the harmful effects of toxicants at ecosystem and population levels.

c) *Toxicant:* Any toxic material or substance is termed as a toxicant. They are hazardous and poisonous. Toxicants are generally man-made and artificial products introduced into the environment due to human activity. They include bisphenol, insecticides and a number of industrial chemicals.

d) *Toxins:* These are produced naturally by living organisms. For example, toxins from the mushroom plant and toxin from the venom of snake are natural toxins.

e) *Poisons:* They are toxicants that cause death or illness in very small doses.

f) *Toxicologists* are scientists who deal with the study of toxicants and toxins.

g) *Xenobiotic* is referred to a foreign substance entering the body. It is derived from the Greek word ‘xeno’ meaning ‘foreigner’.

h) *Toxicosis/ Poisoning/ Intoxication*: Any disease produced by a toxicant.

i) *Tolerance:* The ability of an organism to show less response to a specific dose of a chemical than it demonstrated on a previous exposure; refers to acquired and not innate resistance.

j) *LD 50:* The dose that is lethal to 50% of a test sample or population. Expression of toxicant concentrations is in ppb or ppm in feedstuff, water, air, tissue etc. Other expressions of dose are maximum nontoxic dose, maximum tolerated dose, approximate lethal dose.

1.2.2 Basic Concepts of Toxicology

Both toxins and toxicants are referred to as toxic substances. Toxic substances can be classified as systemic toxins and organ toxins. Systemic toxins have effects on the entire body. An example of systemic toxin include: potassium cyanide. It affects each cell and organ of the body causing complete damage. Some toxicants affect specific tissues or organs. They are known as organ toxins. They do not cause damage to the entire body. An example of organ toxins include: (1) benzene which targets blood-forming tissues and (2) Lead which targets the central nervous system, kidney, and the hematopoietic system.

1.2.3 Historical Development of Toxicology

The historical development of toxicology started very early in human civilizations. Our ancestors who were cave dwellers found plants and animals that had toxins and these toxic extracts were used in hunting and warfare. The earliest medical

works of Hippocrates, Aristotle, and Theophrastus published during 400 to 250 BC include the mention of poisons. Scriptures and records indicate that by 1500 BC hemlock, opium, arrow poisons, and certain metals were also used to poison enemies or for state executions. Some of the notable poisoning victims include: Socrates, Cleopatra, and Claudius. After Renaissance and Age of Enlightenment some basic concepts fundamental to toxicology started to evolve. In toxicology the study of Paracelsus (~1500AD) and Orfila (~1800 AD) are of importance. Advances in toxicology took place during the time of Galen (AD 131–200) and Paracelsus (1493–1541). Paracelsus in his study showed that specific chemicals were responsible for the toxicity of a plant or animal poison. He also reported that the dose of a chemical is an important factor for the human body's response. This is now studies as the dose-response relationship. It is an important concept of toxicology. Hence, Paracelsus was one of the founders of modern toxicology. His well known quote says that "All substances are poisons; it is the dose that makes the poison". Aspirin which is acetylsalicylic acid is a drug consumed by people for medical ailments all over the world. It is relatively safe at recommended doses but chronic use causes deleterious effects on the gastric mucosa, and it is fatal at a dose of about 0.2 to 0.5 g/kg. In the 18th century 'Ramazini's Diseases of Workers' was published in 1700. He is known as the father of occupational medicine. The incidence of scrotal cancer among those working in chimney sweeps was observed by Percival Pott in 1775. Also Hill in 1761 said that nasal cancer and snuff use is correlated. The founder of modern toxicology was Orfila, a Spanish physician who worked at the University of Paris in the early 19th century. He brought out the systematic correlation between the chemical and biological properties of poisons of the time. He analyzed materials for poisons and showed the effects of poisons on specific organs. He was responsible for identifying toxicology as a separate science and he published the first book on toxicology in 1815. In the 20th Century descriptions on the DNA were given and this lead to an advancement of knowledge on toxic effects of substances at the cellular and DNA level. The Silent Spring published by Rachel Carson in 1962 was a landmark publication influencing modern environmental toxicology. Her book served as a catalyst for the establishment of the US Environmental Protection Agency and she is regarded as the mother of the environmental movement. Recently with the advent of molecular techniques in biology detailed studies have been done in toxicology and xenobiotic metabolism.

1.3 SOURCES OF TOXICANTS

Toxic agents can be chemical, physical, or biological in nature and produce toxic effects on the body. The different toxic agents include: chemical (cyanide), physical (radiation) and biological (snake venom). There exist a number of toxicants and they can be classified by various means. Classification may be by exposure classes and by user classes. The different toxicants include the following:

1) *Natural Pollutants:* Toxic pollutants can also be released through natural processes. For example, volcanoes emit particulate matter, sulfur dioxide, hydrogen sulfide, and methane. Forest fires release smoke, unburned hydrocarbons, carbon monoxide, nitrogen oxides, and ash. These can be harmful to human health when inhaled. Dust storms release particulate matter and oceans release aerosols in the form of salt particles. Plants produce pollen and spores, which cause respiratory problems and allergic

reactions.

2) *Anthropogenic Pollutants.* These are pollutants introduced due to human activity/ man-made activities. These substances come primarily from three sources: (1) combustion; (2) industrial; (3) mining and drilling processes.

Let us now learn about some important man-made sources of toxicants in detail. They include the following.

1) *Air pollutants:* Humans have been polluting the air and there are also significant natural pollutants such as terpenes from plants, smoke from forest fires, and fumes and smoke from volcanoes. Among air pollutants there are gaseous pollutants like carbon dioxide, carbon monoxide, hydrocarbons, hydrogen sulfide, nitrogen oxides, ozone and other oxidants, sulfur oxides. There are also fine particulates in the air. The particulates include dust (coal, ash, sawdust, cement), fumes <1 ìm in diameter that come from chemical processes, mist droplets, smoke (0.05–1.0 ìm) resulting from incomplete combustion of fossil fuels and aerosols.

2) *Indoor Pollutants:* These are produced from heating, cooking, pesticides, tobacco smoking, radon, gases, microbes from people and animals. Materials used for construction of buildings can give out gaseous indoor chemicals that have serious health concerns. Carbon monoxide and polycyclic aromatic hydrocarbons released from wood, crop residues, animal dung used for cooking cause acute respiratory infections in poorly ventilated areas.

3) *Water pollutants:* Surface waters may be polluted from point and nonpoint sources. Industrial effluents discharged into waters are an example of point source. Fertilizer and pesticide application in agricultural fields that enter surface waters through rainfall are an example on nonpoint source of pollution. Industrial wastes discharged into waters contain organic and inorganic wastes including hazardous chemicals. Toxic effects are seen in humans when they consume this contaminated water.

4) *Soil pollutants:* When wastes are not properly disposed off then soil also gets polluted. Soil contaminants include: domestic waste, solid wastes, electronic wastes, municipal wastes, agricultural wastes that contain a number of chemicals harmful to life. Further agricultural toxicants like persistent pesticides that do not biodegrade remain in the soil of many years and move into the food chain causing greater health impacts. The most toxic hazardous pesticides are the organochlorine compounds such as DDT, aldrin, dieldrin, and chlordane.

5) *Heavy metals:* Metals released from industrial activities cause toxicity. For example, the heavy metals lead and arsenic are highly toxic and is found in potable water in certain areas. Lead can enter water from lead pipes, lead solder, lead toys, leaded gasoline, utensils and also paints containing lead. Lead is used in cosmetics like lipsticks can regular usage can enter the system and cause health effects. Lead induces neurological damage and can penetrate the placental barrier and induce birth defects among children. Arsenic toxicity is also a serious cause of concern especially in West Bengal in India. It can leach into water from pesticide sprays, arsenic-containing fossil fuels, and leaching of mine tailings and smelter runoff. Chronic high-

level exposures can cause abnormal skin pigmentation, hyperkeratosis, nasal congestion, abdominal pain and cancers. Cadmium enters the food chain through industrial activities. It can accumulate in the tissues of aquatic organisms. Cadmium contaminated rice in Japan caused the disease Itai-Itai. The disease was characterized by severe kidney damage, painful bone and joint problems. Mercury from industries manufacturing plastics, vinyl chloride is also highly toxic to living beings. The Minamata disease in Japan occurred due to consumption of mercury contaminated fishes resulted in neurological disorders, paralysis, and mental disorientation.

6) *Nitrates and phosphate*: These arise from contamination due to fertilizers, discharge from sewage treatment plants, leachate from septic tanks, manure and detergents which are hazardous. They leach into the soil and drinking water. Nitrates in drinking water cause adverse health effects. It occurs due to the formation of: (1) nitrosamine and (2) methemoglobinemia. The nitrates are converted to nitrites by bacteria in the intestine. Thereafter nitrite ions combine with hemoglobin to form methemoglobin. This reduces the oxygen-carrying capacity of the blood and leads to the blue-baby syndrome. This is seen in young or new born children who have ingested nitrate containing water or milk foods.

7) *Petroleum and oil pollutants:* Shore animals, such as crabs, shrimp, mussels, and barnacles, are also affected by the toxic hydrocarbons (oil and petroleum compounds) ingested by them.

8) *Volatile organic compounds (VOCs)*: They include halogenated solvents and petroleum products. They are used in industries involving degreasing, dry cleaning, paint, and in the military. The most important VOCs include: trichloroethylene, toluene, benzene, chloroform, tetrachloroethylene, 1,1,1-trichloroethane, ethylbenzene, trans-1,2-dichloroethane, xylene, dichloromethane, and vinyl chloride. They can move quickly in groundwaters. Exposure to high levels can result in headache, impaired cognition, kidney toxicity, cancer and reproductive disorders.

9) *PCB organic compounds:* These include polychlorinated biphenyls (PCBs), phenols, cyanides, plasticizers, solvents, and numerous industrial chemicals. They are used as coolants in electrical transformers. PCB's are stable, lipophilic, and are broken down slowly only in tissues. Due to these properties they accumulate to high concentrations in fish and waterfowl.

10) *Solvents:* Many types of solvents are used which have systemic toxic effects on nervous system and the blood. Benzene is used in the rubber, canning, printing, shoe manufacturing industries. Benzene affects the hematopoietic tissue in the bone marrow and is an immunosuppressant. Benzene exposure results in decrease of white blood cells, red blood cells and platelets. Continued exposure causes severe bone marrow damage, aplastic anemia and leukemia. Other toxic solvents include: aliphatic hydrocarbons, halogenated aliphatic hydrocarbons, aliphatic alcohols, glycols, glycol ethers and aromatic hydrocarbons.

11) *Asbestos:* This material is used in insulation, roofing for houses, asbestos cements, brake linings, electrical appliances and coating materials. The inhalation of asbestos causes 'Asbestosis', a respiratory disease characterized

by scarring of lungs, fibrosis, calcification, and also leads to lung cancer.

12) *Therapeutic drugs:* Generally all therapeutic drugs are toxic and produce hazardous effects at some dose. This depends on many factors like: dose, nature of the drug, individual (genetic) variation, diet, age, etc. The side effect of chloroquinol, an antidiarrhea drug used in Japan in 1960 caused stiffness of the joints accompanied by damage to the optic nerve. Birth defects or teratogenesis can be caused by drugs particularly, thalidomide. Dermatitis is a common side effect of topically applied corticosteroids. Toxic effects on the blood are caused by chlorpromazine. Hemolytic anemia is caused by methyldopa and megaloblastic anemia is induced by methotrexate. Eye toxicity and glaucoma is caused by thioridazine and systemic corticosteroids. There are many more drugs that cause toxicity to organs.

13) *Drugs of abuse:* They include depressants of central nervous system (ethanol, methaqualone, secobarbital), stimulants of central nervous system (cocaine, methamphetamine, caffeine, nicotine; opioids - heroin, mependine, hallucinogens - lysergic acid diethylamide (LSD), phencyclidine (PCP), and tetrahydrocannabinol, the most active ingredient of marijuana. More important is that these drugs are synthesized illegally and are contaminated with compounds of unknown origin and highly toxic and hazardous to health.

14) *Biological toxicants:* Some naturally occurring substances that cause toxicity include plant, animal, algal, fungal and microbial toxins. They include many phytotoxins and mycotoxins. It is pertinent to understand that a toxin is a toxicant produced by a living organism and is not used as a synonym for toxicant. All toxins are toxicants, but all toxicants are not considered as toxins. Toxins are the metabolic products used for defense against pathogens. These natural products are used in beneficial pharmaceutical purposes and also in biological warfare. For example, Aflatoxins are products of *Aspergillus flavus*. It is fungus that contaminates grain, maize, peanuts, and so on. Aflatoxin B1 is the most toxic and is reported to have carcinogenic effects.

15) *Cosmetics:* Cosmetics induce allergies and contact dermatitis. Lipsticks contain lead at varying concentrations. Hair dyes contain resorcinol which is toxic. In ladies, some of the fibroids are related to regular use of hair dyes. Thioglycolates and thioglycerol used in cold-wave lotion and depilatories have toxic effects. Sodium hydroxide and formaldehyde used in hair straighteners also are reported to exert toxicity. Further paraffin wax is used in some lotions which hinders skin breathing. Parabens and sodium laureth sulfates used in shampoo and body cleansers show toxicity. All these cosmetics are toxic in nature affecting human health.

These were some of the sources of toxicants that have harmful effects on human health. Now let us see the mode of action of some toxicants.

Check Your Progress 1

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) Describe the various sources of toxicants.

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2) Write short notes on (a) therapeutic drugs and (b) drugs of abuse as toxicants.

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3) Define environmental toxicology. Write a brief account of the historical development in toxicology.

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1.4 MODE OF ACTION OF TOXIC SUBSTANCES

1.4.1 Levels of Toxic Action

The mode of action of toxicants can be at the cellular and molecular level and also at the organ level. It depends on the following: (1) uptake, (2) distribution, (3) metabolism, (4) mode of action, and (5) excretion of the substance involved. The different levels and mode of action of toxicants are: (a) *Molecular toxicology*: This includes effects at biochemical and molecular levels. It involves the enzymes that metabolize xenobiotics and the generation of reactive intermediates. It also involves the interaction of xenobiotics with macromolecules and gene expression in metabolic activities inducing toxic pathways. (b) *Behavioral toxicology*: This involves toxic effects on the peripheral and central nervous systems and the endocrine glands. (c) *Nutritional toxicology*: This involves the toxic effects of diet. (d) *Carcinogenesis*: It includes the effects that lead to proliferation of cells in an uncontrolled manner. (e) *Teratogenesis*: It involves chemical, biochemical, and molecular events that lead to toxic effects during development of the foetus. (f) *Mutagenesis*: It involves toxic effects that occur on the genetic material and

the inheritance of these effects causing mutagenecity. (g) *Organ toxicity*: This is the toxic effects at the organ level. They are neurotoxicity - brain, hepatotoxicity - liver, nephrotoxicity - kidney, reproductive toxicity depending on the organ involved.

1.4.2 Sites of Toxic Action

Toxicants can have different mechanisms and sites for initiating their toxic effects. Some of the mechanisms are explained below.

a) *Enzyme Inhibition/Activation*: One important site of toxic action for metals is the interaction with enzymes. It results in enzyme inhibition or activation. Two mechanisms are significant. Enzyme inhibition can occur due to interaction between metal and sulfhydryl groups on the enzyme, or the metal can also displace an essential metal cofactor of the enzyme. For example, in the zinc-dependent enzyme ä-aminolevulinic acid dehydratase, lead can displace zinc thereby inhibiting the synthesis of heme.

b) *Subcellular Organelles*: Toxic metals have the ability to disrupt the structure and function of a number of subcellular organelles. The enzymes associated with the endoplasmic reticulum may be inhibited and metals can be accumulated in the lysosomes. Also respiratory enzymes in the mitochondria can be inhibited and metal inclusion bodies are produced in the nucleus.

c) *Carcinogenic effects:* Some chromium compounds, arsenic, nickel, cadmium cause carcinogenic effects. It may be due to the interaction of the metallic ions with DNA.

d) *Kidney:* It is the main excretory organ of the body and a common target organ for metal toxicity. Cadmium and mercury are potent nephrotoxicants.

e) *Nervous System*: The nervous system is also a common target of toxic organic metal compounds. For example, methylmercury is lipid soluble. It can readily go across the blood-brain barrier and enter the nervous system. Also organic lead compounds are mainly neurotoxicants.

f) *Endocrine and Reproductive System:* The reproductive organs are under neuroendocrine and hormonal control. Hence any toxicant that alters any of these processes can affect the reproductive system. Certain metals can act directly on these organs. Acute exposure to cadmium can cause testicular injury. Toxic exposures to lead can cause testicular degeneration, inhibition of spermatogenesis, and the disease Leydig-cell atrophy.

g) *Respiratory System*: The system can be affected by inhalation of metal dust that can cause irritations and inflammation of the respiratory tract. Chronic exposure can give rise to fibrosis (aluminum dust exposure) or carcinogenesis (arsenic, chromium, nickel metal exposures).

1.5 EXPOSURE ROUTES

The common absorption routes include the: skin, lung and the gastrointestinal tracts. The skin is the largest organ in the human body and is a physical barrier to the absorption of toxic substances. The other major routes are the respiratory

and gastrointestinal tract. These give less resistance to toxicant absorption than the skin. The respiratory tract is the most rapid route for entry and the skin is the least rapid route for entry. This difference is due to thickness of the membranes. Let us now see them in detail.

a) *Skin:* The skin is a complex multilayered tissue. It has a relatively large surface area exposed to the environment. The factors like skin anatomy, physiology, and biochemistry vary among species and within species. So these factors can influence absorption. Our skin is permeable to many toxicants. Dermal exposure to pesticides and industrial chemicals can result in severe systemic toxicity. The human skin is 3 mm thick and the epidermis provides the greatest resistance to toxicant penetration. Most of the systemic absorption occurs at the capillary loops located at the epidermis-dermis junction. Certain other factors like air flow, temperature and humidity etc. can also influence dermal absorption.

b) *Lung:* The respiratory system includes nose, mouth, pharynx, trachea, and bronchus which can reduce the toxicity of airborne particulate substances. There is little or no absorption in these structures. But the cells lining the respiratory tract absorb agents that can cause toxicological effects. The absorption site is the alveoli-capillary membrane that is very thin (0.4–1.5 ìm). It allows for rapid exchange of gases/vapors. The 'residual volume' is the amount of air retained in the lung despite maximum expiratory effort. Hence toxicants may not be cleared out immediately due to the slow release from the residual volume. The rate of entry of some vapor-phase toxicants is controlled by the alveolar ventilation rate. The site of deposition of particles in the respiratory tract is dependent on several factors. They include: aerodynamic behavior of the particles, particle size, density, shape, hygroscopicity, breathing pattern, and lung airway structure. The particle sizes less than 10 - 20 ìm which get through the nasopharyngeal regions and reach the alveoli are medically significant.

c) *Gastrointestinal tract:* This is lined by a layer of columnar cells and protected by mucous. It gives minimal resistance to toxicant penetration. Most of the absorption occurs in the intestine and then in the stomach. Also buccal and rectal absorption can further occur in some cases. Absorption is through passive diffusion mostly, except for nutrients; glucose, amino acids, and drugs. The smaller the particle size of the toxicant, the greater is the absorption. For absorption in this tract the chemical should be in an aqueous form. Bacterial endotoxins, large particles of azo dyes and carcinogens are absorbed by endocytotic mechanisms. Gastrointestinal tract motility is important in the absorption of any toxicant. Excessive rapid movements of the gut can reduce absorption by reducing residence time. Increased blood flow after a meal can result in absorption of several drugs.

1.6 DISTRIBUTION AND STORAGE OF TOXINS IN HUMAN TISSUES

The quantity of toxicant that reaches the target tissue like bone, fat and so on is dependent upon the amount of toxicant absorbed, the distribution in the body, the metabolism and the rate of excretion of the toxic substance. The cell membrane

is selectively permeable. Hence only some substances can pass through. This depends on the molecular weight of the substance, lipid solubility and so on. Most toxic substances pass through cell membranes by diffusion and 75% of the cell membranes are composed of lipids. The electric charge on the toxicant is also an important factor for diffusion. So, non-ionized toxic substances diffuse easily than the ionized ones. Phagocytosis plays an important role in the disposition of particulates that enter the respiratory tract. Asbestos dust and silica dust are absorbed by the process phagocytosis and are engulfed by WBC's in the respiratory tract. Water soluble substances are absorbed in the small intestine by pinocytosis.

a) *Plasma:* In humans the plasma protein binding can vary between and within chemical classes. It is also species specific. The systems of human beings bind acidic drugs more extensively than any other species. Further there are also other variables that can alter plasma protein concentrations. Pregnancy, malnutrition, carcinogenesis, liver abscess, kidney disease, and age can reduce serum albumin. The á1-glycoprotein concentrations can increase with age, inflammation, infections, obesity, kidney failure and stress. These characteristics bring about changes in the body temperature, in the acid-base balance and alter chemical protein-binding characteristics.

b) *Liver and Kidney:* Both these organs have a high affinity for toxic substances and store more toxicants than any other tissue in the whole body. Lipophilic substances like organochlorine pesticides and organic solvents like trichloroethane, methyl chloroform, are readily absorbed in the liver. They can remain in the liver for long periods if they are not biotransformed into water-soluble substances. The liver is a major storage site for water-insoluble toxic heavy metals. Some toxicants can be stored in the liver also. For example, the antimalaria drug quinacrine accumulates in the liver as result of reversible intracellular binding. The concentration in the liver can be several thousand times than that of plasma. In the kidney certain large molecules such as proteins do not easily pass through the walls of Bowman's capsule. Further, unbound metals like cadmium and mercury can be reabsorbed in the cells of the proximal convoluted tubule. Inside the cell they bind to metallothionein resulting in concentration of the toxicants in the kidneys. The kidneys store ten times the amount of cadmium found in the liver, and it can be stored for 10 years or more. Finally bioaccumulation in the kidneys cause complete renal failure.

c) *Lung:* Some particulate material like asbestos, fiberglass toxicants <1µm remain in the lungs. They give rise to respiratory diseases.

d) *Fat tissue:* Toxicants such as lipophilic pesticides, polychlorinated biphenyls and lipid soluble gases accumulate in high concentration in the fat tissue. The adipose tissue (fat) acts as a reservoir for lipophilic persistent organic pesticides, a number of drugs and pollutants. They can sequester these pollutants and then slowly release them into the bloodstream later.

e) *Bone:* Toxicants can be stored in the bone and it can become a reservoir which allows the slow release of chemicals such as lead. Calcium is an important component of bone. Lead can easily replace calcium and is stored in the bones. The effects can be acute or chronic depending on how the

toxicant is suddenly released or mobilized from these depots. Also perfusion of tissues is an important factor for toxicant storage and distribution. The organs like heart, kidney, liver and brain are well perfused than fat and bone. In the fat and bone slow elimination of toxicants occurs. Bone can store toxicants for 10 to 20 years. The toxicants stored in the bone may not be toxic to the bone. It becomes toxic when it is released slowly resulting in nerve damage and so on.

f) *Blood:* The circulatory system and components in the blood stream are primarily responsible for the transport of toxicants to the target tissues or reservoirs. Erythrocytes and lymph also transport the toxicants. Absorption also depends on physiological factors and physicochemical properties of the drug. Hence a reversible movement of toxicants occurs between blood and tissues. Most toxicants pass by simple diffusion down a concentration gradient from the blood to tissues. Tissue mass, blood flow, molecular weight, lipid solubility are also important factors for toxicant distribution. Water soluble toxins are stored here. Also heavy metals like arsenic, thallium, cadmium and chlorinated pesticides, bisphenyl A targets the blood. The toxic substances upon entering the bloodstream bind with plasma proteins such as albumin, transferrin, globulin, and lipoproteins. Most toxic substances are known to bind with the plasma protein albumin.

g) *Passage of toxicants across placenta:* Toxicants can cross the placental barrier and affect the growing foetus by simple diffusion. It occurs readily and easily when the toxicants are lipid-soluble. So the foetus is exposed to almost all drugs even if low lipid solubility drugs are consumed by the mother.

h) *Blood-brain barrier (BBB):* This limits the distribution of toxicants into the central nervous system and cerebrospinal fluid. There are several membranes the toxicant has to cross in order to get into the cerebrospinal fluid. Certain diseases like meningitis disrupt this barrier and allow antibiotics and toxicants to penetrate.

Toxic substances are absorbed and distributed in our body. They are also eliminated, biotransformed and detoxified by the liver and kidneys. Further details will be discussed in the following units.

Check Your Progress 2

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) Describe the various routes of exposure to toxicants.

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2) Where are toxic substances stored in the human body?

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1.7 LET US SUM UP

In this unit we have studied about the various toxicants, their mode of action and sites of action. We have also learnt about the routes of exposure through which toxic substances can enter our healthy body. The toxicants can be absorbed, distributed and also stored in the various tissues and organs of the human body. If the toxic substances are not detoxified or biotransformed, they can remain in the organs causing organ failure and disease. Hence environmental toxicology is a very important aspect that deals with effects of toxicants on human health.

1.8 KEY WORDS

Environmental Toxicology : It is a branch of science that deals with the harmful effects of different physical, chemical and biological agents on living organisms. It is multidisciplinary in nature.

Toxicant **:** Any toxic material or substance is termed as a toxicant. They are hazardous and poisonous. Toxicants are generally man-made and artificial products introduced into the environment due to human activity. They include bisphenol, insecticides and a number of industrial chemicals.

Toxins **:** These are produced naturally by living organisms. For example, toxins from the mushroom plant and toxin from the venom of snake are natural toxins.

Xenobiotic **:** is referred to a foreign substance entering the body. It is derived from the Greek word ‘xeno’ meaning ‘foreigner’.

Toxicosis/ Poisoning/ Intoxication **:** Any disease produced by a toxicant.

Organ toxicity **:** Toxic effects at the organ level. They are neurotoxicity - brain, hepatotoxicity - liver, nephrotoxicity - kidney, reproductive toxicity depending on the organ involved.

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1.10 ANSWERS TO CHECK YOUR PROGRESS

Answers to Check Your Progress 1

1) Your answer should include the following points:

- Air pollutants
- Indoor Pollutants
- Water pollutants
- Soil pollutants
- Heavy metals
- Nitrates and phosphate
- Petroleum and oil pollutants
- Volatile organic compounds (VOCs)
- PCB organic compounds

- Solvents
- Asbestos
- Drugs of abuse
- Biological toxicants
- Cosmetics

2) Your answer should include the following points:
   - therapeutic drugs
   - drugs of abuse as toxicants.
3) Your answer should include the following points:
   - *Environmental toxicology*: It is a branch of science that deals with the harmful effects of different physical, chemical and biological agents on living organisms. It is multidisciplinary in nature.
   - The historical development of toxicology started very early in human civilizations. Our ancestors who were cave dwellers found plants and animals that had toxins and these toxic extracts were used in hunting and warfare.
   - Paracelsus in his study showed that specific chemicals were responsible for the toxicity of a plant or animal poison. He also reported that the dose of a chemical is an important factor for the human body's response. Hence, Paracelsus was one of the founders of modern toxicology. His well known quote says that "All substances are poisons; it is the dose that makes the poison".
   - In the 18th century 'Ramazini's Diseases of Workers' was published in 1700. He is known as the father of occupational medicine.
   - The founder of modern toxicology was Orfila, a Spanish physician who worked at the University of Paris in the early 19th century.
   - Rachel Carson, 1962, Silent Spring.

Answers to Check Your Progress 2

1) Your answer should include the following points:
   - The common absorption routes include the: skin, lung and the gastrointestinal tracts. The skin is the largest organ in the human body and is a physical barrier to the absorption of toxic substances. The other major routes are the respiratory and gastrointestinal tract. These give less resistance to toxicant absorption than the skin. The respiratory tract is the most rapid route for entry and the skin is the least rapid route for entry. This difference is due to thickness of the membranes.
   - Skin
   - Lung
   - Gastrointestinal tract

2) Your answer should include the following points:

- The quantity of toxicant that reaches the target tissue like bone, fat and so on is dependent upon the amount of toxicant absorbed, the distribution in the body, the metabolism and the rate of excretion of the toxic substance. The cell membrane is selectively permeable allowing the passage of some substances depending on the molecular weight of the substance, lipid solubility and so on.
- Plasma
- Liver and Kidney
- Lung
- Fat tissue
- Bone
- Blood
- Passage of toxicants across placenta
- Blood-brain barrier (BBB)

UNIT 2 CHEMICAL TOXICANTS

Structure

2.0 INTRODUCTION

Toxicology is a branch of science which discuss about the source, physical and chemical properties, absorption and pharmacological activity. Chemical toxicology deals with the nature and reactions of toxic substances which involves the origin, exposure and degradation. Any substance whose physiological action gives adverse effects on health is a toxicant. Toxicity of a chemical is determined by many factors like dose, exposure route, and the individual susceptibility (response). Number of synthetic chemicals is produced on the global market and many other chemicals released as by-products. The vital elements like Fe, Cu, Zn, Mo, and Co,V, Mn etc., for biological systems may also show adverse effects above certain concentrations. Elements like As, Sb, Cd, Hg, Be, Al and Pb etc., exist in the atmosphere are not essential for biological system exhibit toxicity even at low concentrations.

2.1 OBJECTIVES

After studying this unit you will be able to:

- classify chemical toxicants;
- define toxicity, toxicant;
- describe various factors of route of exposure;

- describe toxic chemicals at home, food;
- differentiate between domestic and occupational toxicants and
- describe effects of drug and their management.

2.2 CLASSES OF CHEMICAL TOXICANTS

The chemical toxicants categorized under several classes based on their chemical composition and their mode of action. Any condition or disease that results from the exposure to a toxicant is known as toxicosis. The word toxicosis used as an alternative for poisoning or intoxication.

The broad classification of chemical toxicants is 1.Exposure classes 2. User classes.

Exposure Classes: The toxicants under this class are present in domestic and occupational environment. (Ex: food, water, air, soil).

User Classes: This type of toxicants include drugs of abuse, theraupeutic drugs, agricultural chemicals, food additives, metals, solvents and combustion products.

Dear learner we have studied the chemical toxicants in air, water and soil pollution units in course 1.In this unit we will discuss about other toxic exposures in our daily lives.

2.3 EXPOSURE CLASSES

The pollutants under exposure class are air, water and soil pollution has studied extensively in block 2, course 1. However, in this unit we will discuss some important points about them.

2.3.1 Types of Air Pollutants

Air pollutants are classified into following ways. They are:

Gaseous Pollutants: These pollutants are gases and vapors at normal temperature and pressure as well as vapors. The toxic air pollutants of greatest concern are carbon monoxide (CO), hydrocarbons, hydrogen sulfide (H_2S) nitrogen oxides, ozone (O_3), sulfur oxides, and CO_2.The concentrations of pollutants generally expressed as micrograms per cubic meter (ìg/m^3) or for gaseous pollutants as parts per million (ppm) by volume in which 1 ppm = 1 part pollutant per million parts (10^6) of air.

Particulate Pollutants: These are fine solids or liquid droplets that are suspended in air. They exist in various forms. In the form of dust where the particle size is about 100 µm in diameter and released into the atmosphere directly from substances like coal dust, ash, sawdust, cement dust, grain dust. In the form of fumes it exists as suspended solids with size less than 1 µm in diameter, released from metallurgical processes. In the form of mist, it exists as liquid droplets suspended in air with a size of less than 2.0 µm diameter. In the form of smoke it exists as solid particles from incomplete combustion of fossil fuels with a size of 0.05–1.0 µm diameter. In the form of aerosol it exist as a liquid or solid particles suspended in air or in any another gas with a size of <1.0 µm diameter.

2.3.2 Sources of Air Pollutants

Natural Pollutants: Examples of some of the natural pollutants are volcanic eruptions emits particulate matter as well as various gases like sulfur dioxide, hydrogen sulfide, and methane. Huge quantities of pollutants from forest and prairie fires release unburned hydrocarbons, CO, nitrogen oxides, and ash in the form of smoke. Dust storms produce particulate pollutants and aerosols in the form of salt particles produced by oceans. Plants and trees also produce particulate pollutants in the form of produce pollen and spores which cause respiratory and allergic reactions. By the atmospheric reactions with volatile organic compounds released by the trees produce blue haze over forested mountain regions.

Anthropogenic Pollutants: Anthropogenic pollutants released in to the atmosphere from three sources:

i) by burning fossil fuel for heating and power, or exhaust emissions from transportation vehicles that use gasoline or diesel fuels,

ii) industrial processes,

iii) mining and drilling.

The pollutants released from combustion are fly ash, smoke, sulfur, CO, CO_2 and nitrogen oxides. Combustion of coal and oil releases large amounts of sulfur and its oxides that contribute to the formation of acidic deposition. In addition to the fossil fuel combustion automobile exhaust include smoke, lead particles, CO, nitrogen oxides, and hydrocarbons. Industries emit pollutants like sulfuric, acetic, nitric, and phosphoric acids in effluents, solvents, resins, gases like chlorine and ammonia, and metals.

2.3.3 Examples of Air Pollutants

Carbon Monoxide: Once carbon monoxide enters into human body it readily combines with hemoglobin in the blood to form carboxyhaemoglobin, which prevents the transfer of oxygen to tissue thereby affects the cardiovascular function. The CO concentrations e”100 ppm it can cause headaches, dizziness, nausea, and breathing difficulties. Above 700ppm level is always fatal. **Sulfur Oxides** is released in to the atmosphere by industrial combustion of coal because coal containing the highest levels of sulfur. The oxides of sulfur enter the respiratory tract there by create irritation. **Nitrogen Oxides** found in photochemical smog which is also a respiratory irritant that leads to pulmonary edema and hemorrhage. **Ozone is** an oxidizing gas that is formed by photochemical reaction in the atmosphere. The ozone present in the troposphere is harmful and known as bad ozone. Whereas the ozone present in the stratosphere filters the incoming UV radiation known as good ozone. Good ozone is destroyed by chemical compounds like chlorofluorocarbons (CFCs). **Lead** is one of the harmful particulates in air pollutants which impair renal function, nervous system, reduce the development of red blood cells, and impair the nervous system that leads to mental retardation and blindness. Particles like dust and fibers from coal, clay, glass, asbestos, and minerals develop lung fibrosis. The most common disease observed in the coal miners is pneumoconiosis, silicosis is observed in inhaling silica-containing dusts and asbestosis from asbestos fibers are common industrial pollution diseases.

2.4 WATER AND SOIL POLLUTANTS

Surface water is polluted by various point or nonpoint sources. Effluents from an industrial plant or a sewage-treatment plant are a point source and pesticides and fertilizer runoff carried by rainwater into various water bodies is an example of a nonpoint source. Industrial contaminants such as organic waste, solvents, and inorganic wastes like toxic metals pollute the soil and water. In addition industrial accidents may lead to severe local contamination. In addition to the above mentioned pollutants pesticides, fertilizers, detergents, and metals are important pollutants released from urban areas. Perpetual fertilizers and pesticides which are applied directly to the soil in the course of action they move from the soil to the water and make the way to enter the food chain. In another way they leach out of the soil or runoff through rain and flow into the water systems.

2.4.1 Examples of Pollutants

There are various types of pollutants which show toxic effects on environment and human beings. Some of them are discussed in this unit.

Metal Toxicants: Metals toxicants mainly classified into three classes depend on their nature.

1) Metals that are suspected to be a carcinogen,

2) Metals that transport readily in soil, and

3) Metals that proceed through the food chain.

Lead: The sources of lead in water are from lead pipes and lead solder. Lead soil pollution is from seepage of lead from fallout from leaded gasoline and hazardous-waste sites. Lead poisoning has been common in children, particularly in older housing units and inner city dwellings, in which children may consume chips of lead contaminated paint. The toxicity of lead mainly damages hematopoietic system and the nervous system. At low levels of exposure, hyperactivity, decreased attention span, mental deficiencies, and impaired vision is observed in children. At high levels, encephalopathy occurs in both adults and children. Lead damages the arterioles and capillaries which lead to cerebral edema and neuronal degeneration.

Arsenic: Arsenic contamination is due to the leaching of pesticide sprays, released by combustion of arsenic containing fossil fuels, smelter and mine runoff. Toxic level exposures can produce abnormal skin pigmentation, hyperkeratosis, nasal congestion, and abdominal pain. Epidemiologic studies have connected chronic arsenic exposure to various cancers, including skin, lungs, and lymph glands in humans.

Cadmium is released from industrial effluents and enters into the water bodies untreated. This cadmium contaminated water is used for irrigation. The toxicity of cadmium is recognized in Japan after outbreak of the disease Itai-Itai. The people who suffer with this disease have combination of severe kidney damage and painful bone and joint and recognized that occurs in areas where rice is

contaminated with high levels of cadmium. The aquatic organisms can accumulate cadmium in their tissues, leading to increased concentrations in the food chain.

Mercury: Mercury poison is recognized after Minamata tragedy in Japan. In Japan, effluents from a chemical and plastics plant containing mercury were released into Minamata Bay. Bacteria in the aquatic sediments converted the mercury into methyl mercury and that was absorbed by aquatic animals. Consumption of mercury contaminated fish and shellfish by the people suffered with mercury poisoning, or Minamata disease that resulted in death.

Fertilizers: Not only the metal contamination observed in soil and water pesticides are also of major concern. The most toxic pesticides are organochlorine derivatives like DDT, aldrin, dieldrin, and chlordane due to their stability and persistence they can accumulate in food chains.

Fertilizers containing nitrates discharge from sewage treatment plants, and leachate from septic systems and manure leach from soils and enter water bodies. Phosphate fertilizers have a tendency to be absorbed and get accumulated. The increase in the nutrients like phosphates, leads to "algal blooms" or eutrophication, in water bodies. The algal bloom chokes off light penetration and lessens the atmospheric reoxygenation of the water that creates anaerobic conditions and finally death of many aquatic organisms occurs. The adverse health effects from nitrates in drinking water mainly are nitrosamine formation and methemoglobinemea. In human beings by the action of intestinal bacteria the nitrates will be converted to nitrites. These nitrite ions combine with hemoglobin to form methemoglobin, which decreases the oxygen-carrying capacity of the blood and resulting in anemia or blue-baby disease. It is particularly affected to young babies who consume water and milk-formula prepared with nitrate contains water. Some of the nitrosamines are known carcinogens. Oil and petroleum is everlasting pollutants where it is produced by vehicular oil emission or spillage from oil tankers. Oil slicks are very common and responsible for the deaths of many birds in the marine environment. Sea animals like crabs, shrimp and mussels are also affected by the toxic hydrocarbons they consume.

VOC: Volatile organic compounds (VOCs) are a group of halogenated solvents and petroleum products, which are used in large quantities by a many industries, like degreasing, dry cleaning and paint. The properties of VOCs allow them to move quickly into groundwater, and contaminate. The exposure of VOCs can cause headache, impaired cognition, and kidney damage and acute levels of exposure causes cancer, particularly childhood leukemia.

PCBs: Polychlorinated biphenyls (PCBs), phenols, cyanides, plasticizers, organic solvents, and other toxic chemicals that are used as coolants in transformers and are also by-products of the plastic, lubricant, rubber, and paper industries released into the water and contaminates the aquatic animals there by enters the food chain. They are stable, lipophilic in nature and metabolite very slowly in tissues cause tissue damage and responsible for death.

Dioxins: Dioxins are another group of toxic chemical released from industrial accidents and through use of the herbicide 2, 4, 5-T, that contaminates water and soil.

Check Your Progress 1

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) Define the following

i) Toxicity

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ii) Toxicant

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iii) Toxicosis

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2) What are user and exposure class of chemical toxicants?

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2.5 TYPES OF CLASSES

Dear Learners, let us know about types of classes in the following sentences:

2.5.1 Food Additives

Food additives are compounds which are deliberately added to the food products to improve the colour, texture, flavor, appearance and preservation. According to the WHO/FAO joint expert committee, “food additives are non-nutritive substances added intentionally to food, generally in small quantities, to improve its appearance, flavor, texture or storage properties. In view of the safety

requirement Joint FAO/WHO codex Alimentarious commission 1973 has proposed six general principles to be food additive.

1) All proposed food additives must be tested, evaluated toxicologically in all aspects with cumulative, synergetic and potentiating effects.
2) Food additives that are under safe level of intended use will be endorsed.
3) They should be re-evaluated periodically in view of use and safety.
4) Confirmation from CA Commission.
5) Justification should be based on :
    - Preservation and reduction at nutritional quality.
    - Special dietary food products.
    - Monitoring of quality, stability and organoleptic properties.
    - Quality of raw material for food products.
6) Temporary or Permanent approval.

2.5.2 Detergents

1) Detergents are the substances containing molecules which are amphiphilic in nature with a hydrophilic head group and a hydrophobic hydrocarbon tail. The detergents are classified into anionic, cationic, nonionic or amphoteric on the basis of hydrophilic head group. On the basis of chemical characteristics detergents are of two kinds.

2) Phosphate containing detergents

3) Surfactant containing detergents.

- Phosphate containing detergents are highly caustic in nature where as surfactant detergents are very toxic in nature. The phosphates detergents soften the hard water there by suspend dirt in water. The surfactant detergents increase the detergent properties like wetting, foaming, dispersing and emulsifying and helps in removing dirt.

- A large number of surfactant molecules are associated with other components to augment the detergency by which removal of dirt is difficult because of the strong attraction between dirt particles and the fabric but the penetration and adsorption of surfactant molecules onto the and fabric interface becomes very poor.

- Detergents comprise various molecules of surfactants. Surfactants are surface active promoter that is heterogeneous long chain molecule with hydrophilic and hydrophobic components. Surfactants properties like wetting, emulsifying, dispersive, foaming and foaming control ability can be modified by altering the hydrophobic and hydrophilic part. On the basis of this character and ionic (electrical charge) properties in the water surfactants are classified as i. Cationic surfactants ii. Anionic surfactants iii. Nonionic surfactants iv. Amphoteric surfactants.fig.1, table 1.

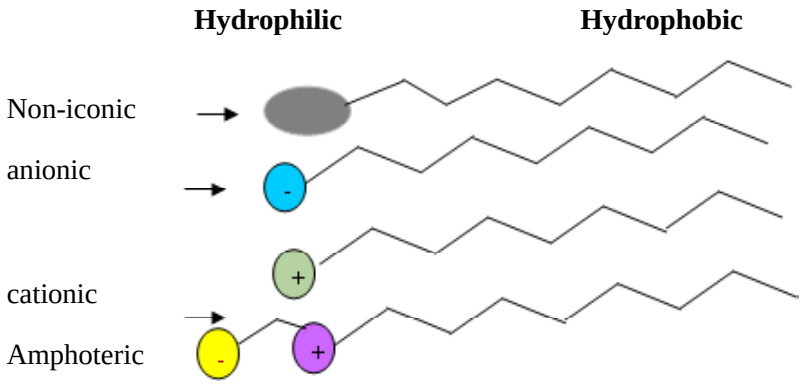

Figure 2.1: Properties in the water surfactants

Cationic Surfactants: Cationic surfactants comprises of a positively charged nitrogen atom and one long chain hydrophobic substituent. Examples are quaternary ammonium compounds with the general formula $R_4N^+X^-$, where X^- is a chloride ion and R is an alkyl group, alkyl trimethyl ammonium chloride, where R contains $C_{8\text{-}18}$ atoms like dodecyl trimethyl ammonium chloride (DDTMAC), dialkyl dimethyl ammonium chloride (DADMAC), and alkyl dimethyl benzyl ammonium chloride. These are commonly used in detergents as softeners.

Anionic Surfactants: Soaps are examples of anionic surfactants comprise of sodium, potassium, or ammonium group. The most commonly used hydrophilic groups are carboxylates, sulphates, sulphonates and phosphates. The anionic surfactants are effectively used to clean oily sand clay l suspension with different degrees on the basis of their chemical composition.

Nonionic Surfactants: This type of surfactants is nonionisable in solution and is effectively used in removing oily soils by the process of emulsification and solubilization. These can be mixed with anionic surfactants for cleaning oil soils. Examples are ethylene oxide, alcohol ethoxylates, alkyl phenol ethoxylates, fatty acid ethoxylates, monoalkanolamide ethoxylates, sorbitan ester ethoxylates, fatty amine ethoxylates and ethylene oxide-propylene oxide copolymers, glycol esters, glycerol esters, glycosides, sucrose esters, amine oxides and sulfonyl surfactants.

Amphoteric Surfactants: Amphoteric surfactants contain both cationic and anionic groups. Examples are the N-alkyl betaines, like laurylamidopropyl-dimethylbetaine.The. Amphoteric surfactants are pH dependent. In acidic solutions, they act as a cationic surfactant, whereas in alkaline solutions they behave like an anionic surfactant. Amphoteric surfactants are also termed as zwitterionic compounds. They are chemically stable and soluble in water and have affinity with other surfactants. The surface activity is depends on the separation of charge between the groups and maximum at the isoelectric point where it exist as zwitterion. Examples are N-alkyl amino propionates, amino dipropionate, alkyl imidazolines.These are also pH dependant. The pH affects their wetting, detergency, foaming abilities.

Along with these the surfactants contain;

Builders- to enhance the cleaning ability. Eg: Citrate,phosphate,sodium carbonate etc.

Antiredeposition Agents: to prevent removed soil from redepositing on cleaned fabrics.Ex: Carboxymethyl cellulose, sodium polyacrylate, polyethylene glycol polymers.

Zeolite: to sequester multivalent metal ions and prevents the anionic surfactants from precipitating out of the solutions.

Alkaline Agents: to remove oily soil containing fatty acids. Ex: Sodium carbonate and sodium silicates.

Processing aids: to provide required physical properties.Ex: sodium sulfate, water, alcohol, xylene sulphonate. Sodium sulfate provides crisp and free-flowing texture; alcohols regulate the viscosity and prevent product separation.

Colorants & Fragrances: to fetch uniqueness to the product especially blue colorants provide a desirable blue/white color to white fabrics. Fragrance conceals the chemical odour of the detergents.

Oxygen bleaches: to remove stain and soil by bleaching action. They contain inorganic peroxygen compounds like sodium perborate tetrahydrate and sodium percarbonate and hydrogen peroxide.

Enzymes: to break down complex dirt molecules, especially proteins like blood and grass. Examples: Protease, lipase and cellulose.

Table 2.1: Properties in the water surfactants

Type	Commercial and domestic examples	Major industrial examples
Anionic	Sodium linear alkylbenzene sulphonate (LABS); sodium lauryl sulphate; sodium lauryl ether sulphates	Petroleum sulphonates; linosulphonates; naphthalene sulphonates, branched alkylbenzene sulphonates; linear alkylbenzene sulphonates; alcohol sulphates
Cationic	Stearalkonium chloride; benzalkonium chloride	quaternary ammonium compounds; amine compounds
Non-ionic	Dodecyl dimethylamine oxide; coco diethanol-amide alcohol ethoxylates; linear primary alcohol polyethoxylate	alkylphenol ethoxylates; alcohol ethoxylates; EO/PO polyol block polymers; polyethylene glycol esters; fatty acid alkanolamides
Amphoteric	Cocoamphocarboxyglycinate; cocamidopropylbetaine	Betaines; imidazolines

Toxicity

The effluents containing surfactants from different sources released into water bodies and shows adverse effects on humans and ecosystems. The degree of toxic effect of surfactants to aquatic plants depends on its concentration. If the concentration of surfactants is high the growth of algae and other microorganisms

in water will diminish slowly, resulting in less productivity of water bodies, thereby impair the food chain of aquatic organisms. This is kind of toxicity increase the rate of membrane permeability thereby disintegrate the structure by material exosmose. In aquatic animals the surfactants enter through skin piercing, gills and animal feeding and thereby enter the food chain. The phosphorous in waste water inhibit the degradation of other toxic constituents. Surfactants stimulate emulsification and dispersion there by reduces the efficiency of sewage treatment. Long term use of surfactant causes skin irritation and allergy. Surfactants like sodium dodecyl benzene sulfonate (SDBS) absorbed by the skin and enter into the blood stream that can cause damage to the internal organs.

Types of Food Additives

According to Federal food, drug and cosmetic Act, five categories of compounds are associated with human food that includes GRAS.

Cat-I : Whose GRAS stature was reaffirmed.

Cat-II : No evidence of toxic hazard.

Cat-III : Additional studies are required for safety.

Cat-IV : Incomplete reaffirm safety. (Evidence of toxicity was reported)

Cat-V : No biological studies available.

Food Colors

Food colors are the natural pigments that are present in the fresh foods which disappear and show toxic effects due to the adverse physical and chemical processing methods. It decreases the visual perception of food product. At about 75% of food products are processed in developed countries. All Food manufacturers replace lost colour and appearance by additives. Food colors are used to restore the original appearance, uniformity and intensity of colour, preserve the flavor and to protect light sensitive vitamins, attractive appearance to colorless gelatin desserts and to identification.

Colorants Subject to Certification

They are very pure chemical with standardized color strengths. The raw materials used are 'Coal tar' dyes or by products of the petroleum industry. Where as certified colors are accessible as water soluble dyes or insoluble pigments. All soluble dyes are available as primary colors or addititive mixtures /with other certified colors.

Colorants Exclude from Certification

They will be classified as natural colors. According to colour additives amendment act of 1960 they or subject to surveillance by FDA.

They are (i) Non-Synthetic (ii) Nature identical (iii) Inorganic colour, which are extracted from animal, plant or mineral source.

Colourants Subject to Certification: The delisted by FDA are:

1) **FD & C Red No.1:** (Ponceau 3R, color index No. 16155). This is a disodium salt of 1 – Pseudocumylazo – 2- naphthol -3,6- disulfonic acid,

which is a dark red color dissolved in H_2O . It is proved to be a liver carcinogen.

2) **FD & C Red No. 2:** Amaranth color index no. 16185. Amaranth is reddish brown powder which is soluble in H_2O gives megenta red or bluish red colour. It is also proved to be a carcinogenetic.

3) **FD & C Red No. 3**: Erythrocine, color index no. 45430 xanthine group of dyes. It is a brown coloured powder and soluble in H_2O that yields red colour and fluorescence with 95% alcohol. Proved to be thyroid tumor, blood and gene mutations.

4) **FD & C Red No. 4**: Ponceau SX, color index no. 14700 originally approved food colour in butter and margarine. It is proved to be chronic follicular cystitis with hematomatoury Projections in to the Urinary bladder, hemosiderotic.

5) **FD & C Red No. 32:** (oil Red Xo, color index No. 12140) It is brownish red powder soluble in oil. Used to colour Oils, Fats, Waxes, Greases, acrylic emulsions, colour the oranges.It is proved to be cathartic,growth retardant,damage to liver & heart tissue.

6) **FD & D Red No. 40:** Allura Red AC. Color index No. 16035 used in cosmetics, drugs and food (soft drinks & cotton candy). Reported to be hyper active agent and growth retardant.

7) **Citrus Red No. 2:** (Solvent Red 80, color index No. 12156) It belongs to monoazodye group. Used to colour the skin of oranges to prepare orange marmalade. Proved to be adenocarcinoma,limphosarcoma and bladder cancer.

8) **FD & C Green No. 3:** (Fast green FCF color index No. 42053). Belongs to triphenyl methane group of dys. Reddish or brownish violet color, soluble in H_2O. It induce sister chromatid exchanges in bone marrow cells and produce sarcoma.

9) **FD & C Blue No. 2**: (Indigotin, Indigo carmine. Colour index No. 73015). It belongs to indigoid family of synthetic dyes soluble in H_2O yielding blue solutions proved to be innocuous and produce tumors at the site of application.

10) **FD & C Yellow No. 3 & 4**: Belong to monoazo group with yellow AB & yellow OB with colour index No. 11380, 11390. They used to colour oleomargarines. Proved to be liver and bladder carcinogens.

11) **FD & C Yellow No. 5**: (Tartrazine, CI no. 19140) orange yellow powder, soluble in H_2O. Proved to be allergic.

12) **FD & C Yellow No. 6**: (Sunset yellow FCF, color index no. 15985). Orange red powder soluble in H_2O gives orange yellow solution. It is proved to be allergic.

Toxicological characteristics of colorants except from certification are Annato extract, Anthocyamius, Dehychated beets, Chlorophyls, Caramel, Tumeric & Cakolene etc.

Acidulate and Sequestrates.

Acidulant are the food additives which are added as preservatives, chelating agent and anti oxidant synergist, flavouring agent's viscosity & melting modifiers and to control pH.

Examples of Acidulant :-

Inorganic Acids: Phosphoric acid and its derivatives, HCl & H_2SO_4.

Organic Acids: Citric acid, benzoic acid, sorbic acid, butyric acid and caprylic acid.

According to the FAO, 0.5% of phosphates are the tolerable level in the diet without any adverse effect. Higher levels may be tolerated if the other ions like Ca, Mg, and K maintained at required levels otherwise it produce adverse effects on physical and chemical characteristics and off flavors as well in food items. The approved dietary intake of phosphorous is <30mg/kg body wt/day in the nutrition of human beings. HCl and H_2SO_4 are not directly used as an acidulant but used in hydrolysis of proteins, starch and corn syrups. HCl & H_2SO_4 are corrosive to all body tissues. Inhalation causes lung damage and skin contact results in necrosis.**Vinegar** is a aqueous solution of acetic acid. It is used as acidifier, flavor enhancer, pickling agent and pH controlling agent. It is absorbed in the gastro intestinal tract and used up in oxidative metabolism, formation of glycogen intermediates of carbohydrates and synthesis of fatty acids and cholesterol. Acetic acid in H_2O or organic solvents is strongly corrosive to the skin causes tissue damage and produce canker sores. Lactic acid is present in pickles, beer, buttermilk and cheese. It is used as acidifier, antimicrobial agent, curing agent, flavoring and carrier agent. According to FAO/WHO the permissible limit of D (-) isomer is 100mg/kg/body/day.

Adipic Acid: It is one of the most important of aliphatic dicarboxylic acid used as leavening agent, neutralizing and flavoring agent. The permissible limits are.

Baked items – 0.05%

Non-alcoholic beverages – 0.005%

Condiment and relishes – 0.5%

Dairy products – 0.45%

Fats & Oils – 0.3%

Frozen dairy desserts – 0.0004%

Gelatin & puddings – 0.55%

Meat products – 0.3%

Above these limits it causes intestinal hemorrhage.

Allyl isothiocyanate: It is used as spice seasorings and condiments. It is formed from sinigrin by crushing the moistened mustard seeds.It is proved to be skin irritant and at high concentrations results in epithelial hyperlasia and uleers.

Cinnamyl Anthranilate: Is a synthetic flavoring agent used to provide grape or cherry flavor to beaverages, candy, puddings, and chewing gum at 1000 pm concentrations. Above this limit it is thought to be carcinogenic.

Menthol: It is a synthetic and natural constituent of peppermint oil used as flavoring agent in candies, chewing gum. It can cause sensitization reactions like urticaria. At high concentrations it causes heart fibrillation.

Monosodium Glutamate (MSG): MSG is liberally used in flavor enhancer in meat products. It provides Umami sensation generally called as a 5^{th} basic taste. At high concentrations, it causes Chinese restaurant syndrome (CRS) in sensitive population with symptoms like upper body tightness, warmth and feeling pressure.

Myristicin: Nutmeg oil, mace oil contains < 4% Myristicin. It has psychomimetic and narcotic properties. It is thought to cause headache, abdominal pain and nausea at high doses. At elevated levels cause liver damage & death.

Yeast: Yeast is used to ferment the baked food items. At high levels in food products causes high uric acid levels in blood. The urate oxidase enzyme is absent in human beings. During the fermentation of yeast, pharmacologically active amines and tyramine formed which are responsible for higher BP.

Parabenes: Parabenes are the esters of p-hydroxy benzoic acid (PHB) used an antimicrobial agent in food products, cosmetics and also known as paracepts or PHB esters. These are used in malt beverages and non carbonated soft drinks. At high concentrations, it can causes dermatitis.

Polycyclic aromatic hydrocarbon (PAH): PAH enters into the human food chain by (i) Polluted air on food crops. (ii) Heat processing of foods like roasting, smoking and grilling. iii.Preparing the food products above 400 ^{0}C resulting in significant formation of PAH like benzo [α] pyrene, dibenzo (α,β) anthraene, dibenzo [α,β] pyrene and benzo[α] fluro anthrene. All these compounds are potent carcinogens.

2.5.3 Cosmetics

Cosmetic products are the substances to be applied to the external parts of the human body including teeth and oral cavity for cleaning purpose, appearance, protecting and maintaining in good condition. Cosmetics are classified into two types.

1) Leave-on 2) Rinse-off

Cosmetics which are leave-on category can be intended to lasting for certain period on the skin like perfumes, cosmetics used for decoration, body and face creams. A rinse-off cosmetic is a product one which should be rinsed off after a period of time like shampoos, soaps, shower gels, ointments and toothpastes.

Recent years, cosmetics, and many other beauty products for personal care that do not fall within cosmetic regulation are noticed as emerging pollutants because they are constantly released into the terrestrial and aquatic environment. They are found to be persistent, bioactive, and bio-accumulate with potential adverse ecological and environmental impact. Substances like perfluroalkyl compounds, parabenes, organic UV filters and microplastics.

1) **UV Filters**

Cosmetic products like sunscreens and skin lotions contain benzophenones (BPs) have ultraviolet (UV) filter properties which absorb UV-A (315–400

nm) and UV-B (280–315 nm) radiation. They also used as additive in plastics, printing inks, shampoos, perfumes and photographic films to prevent UV light damage. The Benzophenones are highly lipophilic in nature and bioaccumulate in the human body by crossing dermal tissue. Studies proved that BP UV filters could be detected in the plasma, bile and urine after application. These filters also found in the surface waters there by enter in to the food chain. The concentration of BPs in sewage sludge exceeds 10 mg/kg of dry matter proved to be mammary cancer cell proliferation. The photo stability of UV filters plays an important role to absorb UV light.

2) **Inorganic Filters**

The inorganic filters present in sunscreen are TiO_2 and ZnO. The nano TiO_2 and ZnO particles are almost customarily present in sun blocker formulations because they nanoscale enhance skin retention, acceptance for consumers, and the UV depletion properties. By the process of immersion or abrasion after application these compounds released into the environment. The sewage treatment plants (WWTPs) expel most of the TiO_2 present in the sewage, but a small amount is released into natural water bodies where it aggregate and remain in suspension. The residues of TiO_2 released from sunscreen form aggregates submicron level which remains in suspension in fresh water, and in water bodies with high salt concentrations like sea they aggregate and progressively settle down and detained in the sediment.

By the photocatalytic reactions of UV radiation the nano TiO_2 and ZnO generates reactive oxygen species likes OÅ”²", Å”OH, and H_2O_2. The photoactivity of these species causes cellular damage and environmental toxicity. To prevent the production of ROS, TiO_2nanoparticles present in sunscreen are coated with silica and alumina and/or doped with manganese or vanadium, even though sometimes these coatings do not withstand contact with water.

These results suggest that the normal recreational activities in coastal resorts can result in the production of significant amount of H_2O_2 and consequent damage to or death of marine coastal phytoplankton; this could have reverberations on the marine food web, which relies on these microorganisms. The ZnO is extremely toxic to the aquatic animals like zebra fish, marine algae and sea urchins.

3) **Parabenes**

Parabenes are alkyl esters of the *para*-hydroxybenzoic acid which are used as preservatives due to their antimicrobial activity against yeasts, molds, and bacteria and their chemical stability, low toxicity, and low cost. They are extensively used in cosmetics, including powders foundations, , lipsticks, eye shadows, mascara, lip glosses and nail polishes, and in pharmaceuticals and personal care products such as lotions, sunscreens, cleansers, shampoos, deodorants, hair care products, and toothpaste. They are proved to have the potential to produce contact dermatitis, irritation, or photo contact dermatitis.

4) **Triclosan**

Triclosan (TCS), 5-chloro-2-(2, 4-dichlorophenoxy) phenol is a preservative used personal care products like hand soaps, shampoos, detergents,

toothpastes, sunscreen, and deodorants. The domestic sewage contains most of the TCS and enters into the aquatic environment in spite of the treatment. During sewage treatment TCS is converted into chlorinated derivatives that are more persistent and toxic to the aquatic life. It also enters into the terrestrial environments because of the sewage sludge application on farm land as a fertilizer. TCS is stable and lipophilic in nature due to which it bioaccumulate in algae, plants, earthworms, marine mussels, snails, amphibian larvae, fish, and marine mammals causes adverse ecological effects and alters benthic bacterial community composition, exhibits teratogenic effect. TCS has potential to impair thyroid function, endocrine disruption, oxidative stress, and liver carcinogenesis.

5) **Plastic Microbeads**

Plastic microbeads are pieces of plastic, spherical in shape, size varying from <mm-1mm. They are widely used in soaps, face wash, toothpaste, exfoliating scrubs and anti-ageing creams because exfoliating debris from the skin by replacing natural exfoliating materials like pumice, oatmeal, apricot husks. The microbeads are made by different types of plastic material. They are

Polyethylene (PE), polymethyl methacrylate (PMMA), Nylon, Polyethylene terephthalate (PET) and Polypropylene (PP).

When these products are washed down after use the microbeads in the products pass through sewage systems. Because of nonbiodegradable nature and very small size they enter into rivers and canals and finally into the sea and ocean that contribute to the plastic pollution. Because of the small size and large surface area plastic microbeads absorb POPs and other pollutants in aquatic environment. Aquatic species consume these microbeads by mistake since they are not able to distinguish between food and microbeads there by enter into the food chain and regularly consumed by people. The harmful toxic chemicals which are added during the manufacture of plastic like plasticizers and flame retardants which are drain out into water bodies and produce adverse effects by polluting them. These plastic microbeads block the intestine in marine animals, impair reproductive ability and reduce the growth rate.

Check Your Progress 2

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) Define food toxicology

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2) What are the types of food additives?

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2.6 KEY WORDS

Acute Effect : Effect of finite duration occurring rapidly (usually in the first 24 h or up to 14 d) following a single dose or short exposure to a substance or radiation

Additive Effect : Consequence that follows exposure to two or more physicochemical agents which act jointly but do not interact: The total effect is the simple sum of the effects of separate exposures to the agents under the same conditions

Adverse Effect : Change in biochemistry, physiology, growth, development morphology, behavior, or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to other environmental influences.

Air Pollution : Presence of substances in the atmosphere resulting ither from human activity or natural processes, in sufficient concentration, for a sufficient time and under circumstances such as to interfere with comfort, health, or welfare of persons or to harm the environment.

Bioaccumulation : Progressive increase in the amount of a substance in an organism or part of an organism that occurs because the rate of intake exceeds the organism's ability to remove the substance from the body.

Contaminant : 1. Minor impurity present in a substance. 2. Extraneous material inadvertently added to a sample prior to or during chemical or biological analysis 3. In some contexts, as in relation to gas cleaning equipment, used as a synonym for "pollutant", especially on a small scale. 4. Unintended component in food that may pose a hazard to the consumer

Detergency : The term 'detergency' is used to describe the process of cleaning by surface active agent. Detergency can be defined as removal of unwanted substance (soil) from a solid surface brought into contact with a liquid. The word 'soil' in connection with textile surfaces most frequently

denotes the unwanted accumulation of oily and/or particulate materials on the surfaces or interior of fibrous structure

Poison : Substance that taken into or formed within the organism impairs the health of the organism and may kill it.

Photostability : The term photostability means resistance to permanent structural and functional changes under the infl uence of solar energy.

2.7 LET US SUM UP

Chemical toxicants are released into the environment in different ways, and they can transport through many pathways and show adverse effects on the environment. A toxicant present in the environment at a given point in time, it can be stationary, it can be transported, or it can be transformed into another chemical species. The life cycle of a chemical depends on the physicochemical properties, characteristics of the environment to which it is released. The chemical toxicants released into the atmosphere exerts adverse effects on humans and other terrestrial and aquatic organisms like intentional ingestion, occupational exposure, environmental exposure, as well as accidental and intentional poisoning.

2.8 REFERENCES AND SUGGESTED FURTHER READINGS

Hemond, H.F., and E.J.Fechner. Chemical fate and transport in the environment. New York Academic Press,1994

Mackay, D.,W.Y.Shiu, and K.C. Ma.Physical-Chemical Properties and environmental fate and degradation hand book, CRC Press 2000.

Rand, G.M., ed. Fundamentals of aquatic Toxicology:Part II Environmental Fate,Washington DC;Taylor and Francis,1995.

2.9 ANSWERS TO CHECK YOUR PROGRESS

Answers to Check Your Progress 1

Your answer should include the following points.

1) a) Toxicity is the amount of poison that, under a specific set of conditions, will cause a detrimental effect (agents are usually compared on a mg/kg basis, toxicity is not the condition produced by the toxicant).

   b) Toxicant: Any substance whose physiological action gives adverse effects on health is a toxicant.

   c) Toxicosis: It is the condition or disease state that results from exposure to a toxicant. The term toxicosis is often used interchangeable with the term of poisoning or intoxication.

2) Exposure classes are the toxicants under this class are present in domestic and occupational environment. (Ex: food, water, air, soil).Where as User classes are the type of toxicants include drugs of abuse, therapeutic drugs, agricultural chemicals, food additives, metals, solvents and combustion products.

Answers to Check Your Progress 2

Your answer should include the following points.

1) It deals with natural contaminants, food and feed additives, and toxic and chemo-protective effects of compounds in food. Food Toxicology is involved in delivering a safe and edible supply of food to the consumer. During processing, a number of substances may be added to food to make it look, taste, or smell better. Fats, oils, sugars, starches and other substances may be added to change the texture and taste of food. All of these additives are studied to determine if and at what amount, they may produce adverse effects. A second area of interest includes food allergies. Almost 30% of the American people have some food allergy. For example, many people have trouble digesting milk, and are lactose intolerant. In addition, toxic substances such as pesticides may be applied to a food crop in the field, while lead, arsenic, and cadmium are naturally present in soil and water, and may be absorbed by plants. Toxicologists must determine the acceptable daily intake level for those substances

2) According to Federal food, drug and cosmetic Act, five categories of compounds are associated with human food that includes GRAS.

   Cat - I - Whose GRAS stature was reaffirmed.

   Cat – II - No evidence of toxic hazard.

   Cat – III - Additional studies are required for safety.

   Cat – IV - Incomplete reaffirm safety. (Evidence of toxicity was reported)

   Cat – V - No biological studies available.

UNIT 3 BIOLOGICAL TOXICANTS

Structure

3.0 INTRODUCTION

The term toxin was first used by organic chemist Ludwig Brieger. Biological toxins are unique biological molecules that are mainly used for protection and predation which affect cells or organs or an entire system of a target species. Most commonly all biological toxins are proteins and especially are capable of modulating biological process by a number of ways, interacting with the biological receptors and blocking their activities. Some of the toxins are nonspecific, but most of them have the target specificity. Biological toxins are mainly biological agents which involve bacterium, virus, protozoan, parasites or fungus. There are about 1,200 different types of potential bio agents. Biological toxins are toxic substances which are produced by plants, animals and microorganisms that can cause harmful effect when ingested, injected, inhaled or absorbed. Biological toxins may be stated as "any toxic substances occurring in nature produced by animals, plants or microbes (pathogenic bacteria), such as bacteria, fungi, flowering plants, insects, fish, reptiles or mammals."

3.1 OBJECTIVES

After examining this unit, you must be able to;

- give a list of different type of biological toxicants;

- discuss suitable examples of bacterial, viral and fungal toxins;
- list the diseases caused by the biological toxicants;
- describe the measures of bacterial, viral and fungal toxicants;
- discuss the various types of virulent and prevalent biological agents;
- describe the classification of Toxicants present in food and
- give an outline of preventive measure by biological agents and relevant measure that can be used to control exposure.

3.2 TYPES OF BIOLOGICAL TOXICANTS AND FOOD INTOXICATION

Biological toxins are poisonous substances produced by living organisms from emergent microscopic species to well-developed species. Normally, toxins are neither dermal nor considered a volatile exposure hazard. Toxins are usually much more noxious than any of the other chemical agents. Characteristics of selected biological toxins are shown in Table 3.1.

Table 3.1: Characteristics of Selected Biological Toxins (Thomas *et al.*, 2000)

Source	Toxin	LD_{50} (µG/kg)	**Required Detection Capability**[a]	**Notes**
Fungus				
Fusarium species	Trichothecenemy-cotoxins (‘’yellow rain”)	25 to 500 (inhalation)	40 mg/m3 (air)	Nonlethal and delayed effects.
				Inhalation, ingestion, dermal.
		1600 oral	40 ppm	Very stable
				repeated small doses are cumulative
Bacteria				
Clostridium botulinium	Botulinium A, B, C, D, E	~ 0.02 (inhalation)	0.1 mg/m^3	Comes under most potent Known toxins
		1 (oral)	0.02 ppm (water or food)	Delayed Lethaleffect .
				Persists in food and water.
				It takes around 12 hoursin air for its break down in.

Clostridium perfringens	Gangrene-causing enzyme	0.1 to 5	0.3 mg/m^3	Delayed action.
				Low mortality, but very debilitating.
Clostridium tetani	Tetanus toxin	~ 3	N/A	Delayed action.
				Heat sensitive and unstable.
Cornyebacterium diptheria	Diptheria toxin	0.03	N/A	lethal.
				Rapid acting.
Staphylococcus aureus	Staphylococcus enterotoxin A, B, C, D, E (Toxicity is for type B)	0.4 (aerosol ED_{50})	0.058 mg/m^3	Rapid acting.
		0.3 (oral ED_{50})		Severely incapacitating.
		20 (aerosol LD_{50})		Symptoms persist for almost 24 to 48 hours
			3 mg/m^3	Can be lethal.
				Large-scale production feasible.
			0.007 ppm	Very stable.
Plants				
Ricinus communis	Ricin	1,000	150 mg/m^3 (air)	Lethal, delayed action.
			20 ppm (water)	Easily produced.
				Persistent
Animals				
Palythoa (soft corals)	Palytoxin	0.08 to 0.4	0.006 ppm(water)	Stable
			0.035 mg/m^3 (air)	Lethal and rapid acting.
Conus magnus (fish-hunting cone snails)			~0.1 ppm (water)	Highly stable.

Easily synthesized.				
Conusgeo-graphus	Conotoxins	3 to 6	~0.6 mg/m^3 (air)	mostly used as aerosols.
Phyllobatesa-urotaenia and *Phyllobates-terribilis*	Batrachotoxin	0.1 to 0.2	0.015 mg/ m^3 (air)	Water soluble. Rapid acting and lethal.
				Very stable.
				Can be synthesized.

[a] Assumption : (For 70 Kg adult person) Air= 0.016 m^3/min for half hour breathing, water= 3 Lingestion or Food: 3 kg ingestion.
[b] IP = intraperitoneal injection doses to mice.

Dear Learners, let us now learn about types of biological toxicants in the following sentences:

3.2.1 Types of Biological Toxicants

- **Aitoxin**: It is honey bee venom and it is injected via the sting.
- **Cyanotoxins**: These toxins are produced by cyanobacteria.
- **Cytotoxins**: These are the substances which are toxic at the cell level (kills individual cells).
- **Haemotoxins**: These substances are carried in bloodstream and target red blood cells.
- **Mycotoxins**: These toxins are produced by various fungi.
- **Necrotoxins**: These substances cause tissue destruction via cell death and are carried in the bloodstream.
- **Neurotoxins**: These substances affect the nervous system.

3.2.2 Food Intoxication

Food intoxication is a type of food-borne illness which is caused by ingesting exotoxins formed by organisms such as fungi, bacteria or other microbes or by having the food which is toxic in nature for animals or humans. Food intoxication can lead to illness very rapidly and person can become very sick. About 300,000 people are reported to be hospitalizations every year and the mortality rate is around 2000-4000 person.

Microbial food borne illnesses or food poisonings mainly falls into one of two categories that is Food Poisoning Infection vs. Food Poisoning Intoxication. The first is food infection; in this case the microorganism itself grows inside the body. The second is food intoxication, where a chemical or natural toxin is present in the food. Most of the bacterial food poisonings are mostly food infections. Symptoms and Signs may start after few hours of intake of contaminated food,

or symptoms may begin after few days or even weeks later. Sickness due to food poisoning generally persists from a few hours to several days.

3.3 CLASSIFICATION OF TOXICANTS PRESENT IN FOOD

1) **Naturally Occurring Toxicants**

2) **Toxicants from Microorganisms**

3.3.1 Naturally Occurring Toxicants

Carbohydrates, sugars, proteins and vitamins are natural chemicals obtained from food that are essentials for growth and health. But some food items contain natural toxins which are potentially harmful. The cause for the presence of natural toxins is always not known. In some food items, a toxin is available as a naturally occurring pesticide to protect them from insect attack.

i) Lathyrism

- This is a crippling disease accompanied by paralysis of the leg muscles occurring mostly in adults who consume large quan-tities of the seeds of *L. sativus* or other lathyrus species for a long period

ii) Ackee Fruit Poisoning

- The poisonous properties of the fruit are due to unusual amino acid, hypoglycin A, B. Both have strong hypoglycemic action resulting in coma and death.

iii) Goitrogens

- Many food stuffs contain organic com-pounds which have goitrogenic proper-ties.
- The red skin of groundnut contains phe-nolic glycosides which possess goitrogenic properties.

iv) Pressure Amines

- The pressure amine foods are mainly plan-tains (green and ripe), juices of pineapple and tomato, banana, lemon etc.

v) Argemone Seed Oil Poisoning

- The toxic substance in argemone seed is sanguinarine.

vi) Fruit Seeds and Pits

- Inner stony pit (kernel) of peaches and apricots and seeds of apple and pear contain a naturally occurring substance known as amygdalin. Amygdalin causes discomfort or illness by releasing hydrogen cyanide in the gut. It can be lethal if consumed too much in a short duration of time.

Check Your Progress 1

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) What do you understand by biological Toxicants?

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2) What are the types of Biological Toxicants?

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3) What is food intoxication? Give the classification of toxicants in food.

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3.3.2 Toxicants from Microorganisms

Anthrax : It is an infectious disease showing acute nature of onset which is caused by a spore-forming bacterium known as *Bacillus anthracis*. It is generally acquired after being in contact with any anthrax-infected animals or by anthrax-contaminated animal products.

Avian Flu : Avian influenza is a disease of birds which is highly contagious and is currently epidemic amongst poultry in Asia. Despite the uncertainties, poultry experts agree that immediate culling of infected and exposed birds is the first line of defense for both the protection of human health as well as for the reduction of further losses in the agricultural sector.

Hantavirus : Hantaviruses are mostly transmitted to humans from the dried droppings, urine or saliva of mice and rats. Workers who work in animal laboratory and in infested buildings are at higher risk to this disease.

Molds : Molds produce and release millions of spores which are small enough to be air, water, or insect-borne which may have negative impacts on the human health such as asthmas, allergic reactions and causes other respiratory problems.

Mycotoxins : Mycotoxins are metabolites of fungus. Some of the mycotoxins are highly toxic to the animals and are potentially toxic even to human beings also. Recently concern is related to their presence in many food and theiritems carcinogenic properties.

Patulin : Patulinis mostly produced by the *P. expansum*. *P. expansum* is related especially with a range of moldy vegetables and fruits, in particular figs and rotting apples. It is not available in apple beverages, like cider because it is destroyed by the fermentation process. It is reported that the damage of immune system of animals has been reported but it has not shown to be carcinogenic.

Penicillium Islandieum : The yellow discol-ouration in rice has been reported in Japan by the contamination of rice by *Penicillium islandieum* during storage and develops toxic symptoms in man.

Plague : World Health Organization reported that1,000 to 3,000 cases of plague are reported every year. A bioterrorist release of plague can cause rapid spread of the pneumonic form of the disease and this will have devastating consequences.

Smallpox : It is a highly contagious disease which is unique to humans. It is estimated that not >20 percent of the population has any immunity from the previous vaccination.

Tularemia : Tularemia is well known as “deer fly fever” or “rabbit fever” and it is extremely infectious. It is an attractive weapon for use in bioterrorism because relatively only few bacteria are required to cause the disease.

3.4 MICROBIAL AGENTS: SYMPTOMS, EFFECTS ON HEALTH AND MANAGEMENT

Dear Learners let us now learn about the various microbial agents which can act as a biological toxicants, it can be a bacterium, virus, protozoan, parasite, or fungus that can transmit infection ranging from mild to severe, and in some cases may even be life-threatening. They affect blood, skin, gastrointestinal tract or any organ of the body. Therefore, it is important to know their symptoms and how to prevent infections caused by them.

3.4.1 Bacterial Agents

They are defined by its virulence or ability to induce disease, mode of transmission incubation period, and case mortality rates. Some bacteria form spores when they find themselves in a hostile environment. The bacterium replicates its genetic material, through a process known as sporulation, and then it surrounds it by a thick coating. The bacterium released its water and its metabolism ceases in the spore form. In spore form the bacterium can survive in extreme temperature,

radiation, and lack of nutrients, air, and water for extended periods of time and they are revived again when nutrients are abundant. A spore is ideal as biological weapons agent due to its virtual indestructibility.

a) *Salmonellosis*

Salmonella food poisoning results from ingestion of any food containing appropriate strains of this genus in significant numbers. The salmonella are gram negative small, non-spore forming rods that are indistinguishable from Escherichia coli under the microscope or ordinary nutrient media.

Symptom/Effects

The salmonella has incubation period of about 12-36 hours. Salmonella does not release any direct toxin product rather the bacterium induces the responses in the infected animal, this may result in appearance of symptoms. The clinical signs includes diarrhea, which may be watery, greenish and foul smelling. Other findings include muscle weakness, moderate fever or prostration. The main symptoms are gastrointestinal, which includes nausea, vomiting, bloody diarrhea with mucous or abdominal cramps with possibility of rose spots. In young children and in older age .These symptoms can be more severe. In most of the cases symptoms resolves within 2-3 days without any complications.

Management

There is an urgent requirement to develop new methods to control the food poisoning and spoilage by salmonella, ordinary farms by instituting bio-security and bio-containment practices in addition to enhanced food storage practices, food preparation and processing method. Effective heat processing of animal origin food products includes pasteurization of milk and eggs, poultry thermal processing and irradiation of meat; vaccination of food producing animals and egg-producing flocks, following good hygiene practices during food production. Food service establishment, safe food preparation practices, which includes through cooking, boiling of milk and reheating of food, adequate refrigeration, prevention of cross contamination of food; cleaning and disinfection of food preparation surfaces; exclusion of pets and other animals from food handling areas are good practices. The vulnerable group person should avoid raw milk, eggs or under cooked meat and poultry products, and foods containing raw egg or unclean vegetables.

b) *Staphylococcus aureus*

Saureus is gram positive cocci. It occurs in the form of singles, tetrads, short chains and irregular grape like clusters. Only those strains of *Staphylococcus* which produces enterotoxins can cause food poisoning. The one who handles the food with an active lesion or carriage later they initiate the infection.

Symptom/Effects

The characters of food poisoning caused by *Staphylococcus aureus* appear in around 2-4 hours, because of shorter incubation period of bacteria. The onset of symptoms is sudden and is characterized by vomiting and diarrhea without any fever. This illness can remains for less than 12 hours. In severe cases masked pallor, dehydration and collapse may need treatment including intravenous infusion. The intoxication is characterized by short incubation periods where illness is the results of ingestion of the toxin in the food.

Management

Food borne bacterial illness by bacteria can be commonly controlled and prevented by proper cooking as well as storing. For example adequate refrigeration of food, personal hygiene, adequate cooking and heating are important. The control measures also include; a) educating people who prepare or handle food, so that they can have proper measures; b) prohibiting persons from handling food who have skin lesions; c) keeping food at 4 degree centigrade or lower to prevent bacterial multiplication and the formation of toxin. Food items should not be left at room temperature for longer times.

c) ***Clostridium botulinum***

It is a gram positive, anaerobic and spore bearing bacilli, which has wide distribution range in soil, decaying vegetations, sediments of lakes and ponds. Seven different strains of the organisms (A-G) are classified based on serologic specificity and other neurotoxin. Most reported human outbreaks are associated with fish and sea food products.

Symptom/Effects

C. botulinium have 12 - 36 hours of incubation period. The most common features include vomiting, thirst, dryness of mouth, constipation, ocular paresis (blurred-vision), difficulty in speaking, breathing and swallowing. Death occurs due to respiratory paralysis within 7 days. Clinically, botulism recognized as a lower motor neuron disease resulting progressive flaccid paralysis.

Management

Preformed toxin in food can completely destroyed by exposure to a temperature of 80^{o}C for 30 minutes or boiling for 10 minutes. Therefore all homo canned low acid foods should be boiled before tasting for consumption. Never taste food if it has an odor shows gas formation. Prevention of food born botulism also depends on ensuring effective control of commercially and home canned foods are destroying all *C. botulism* spores. This requires cooking at 121°C or higher. Home canned vegetables should be boiled and stirred for at least 3 minutes prior to serving to destroy botulism toxins. Foods with apparent off odors or suspected odor should not be opened.

d) ***Clostridium perfringens***

Clostridium perfringens is anaerobic spore bearing,gram-positive bacilli that is present abundantly in the environment, vegetation, sewage and animal feces. Organisms which produce type A enterotoxins commonly causes perfringens food poisoning.

Symptom/Effects

The incubation period of *Clostridium perfringens* is about 8-24 hours. The illness is characterized by acute abdominal pain, vomiting and diarrhea. The classic symptoms of *C. perfringens* type A food poisoning are diarrhea with lower abdominal cramps. Vomiting is not common, and fever is rare. Symptoms typically occur within 8-24hours after ingestion of temperature abused foods containing large number of vegetative cells of the bacteria. The illness is self-limiting and the patient recovers within 8-24 hours.

Management

The *C. perfringens* gastroenteritis syndrome often occurs in institutional cafeterias thus giving proper attention towards the leading causes of food poisoning can prevent bacterial intoxication. Cooking at temperatures below 100°C will allow the survival of the spores. The cooking process blocks oxygen, creating real anaerobic conditions in foods like pies, gravies, strew and rolls of cooked meat and in poultry carcass. Therefore, prevention of vegetative cells can be done by cooking food properly and this is the most practical way of preventing *C. perfringens* food borne illness.

e) ***Escherichia coli***

Certain strain of E. *coli*shows characteristics of hemolytic activity on blood agar. It is motile with peritrichous flagella and often fimbriae.

Symptom/Effects

The incubation period of Escherichia coli about is 72-120 hours. The clinical symptoms initially may be diarrhea including abdominal cramps, which may turn into grossly bloody diarrhea in a few days. There is however, no fever. The symptoms of E. coli septicemia are mainly referable to bacteremia, and toxemia where the effect of bacteria localization is seen in variety of tissue spaces throughout the body.

Management

The prevention of food borne illness caused by *E.coli* can be the same as thatof other food borne illness caused by bacteria. However the consequences in young children may be more and special precaution is needed. The sensitivity of this organism is such that cases should not occur when food properly cooked. In the cases of ground beef, the recommendation is that it should be cooked to 160°F or the core temperature should be brought to a minimum of at least 155°C for at least 15 second and that the juices are clear. Once cooked, the food meals should not be held for more than 3-4 hours at temperatures between 40°F to 140°F.

3.4.2 Fungal Agents

Fungi are found almost everywhere. Approximately 1.5 million different types of fungi species are found on the Earth, out of which only about 300 have the capability of making people sick. Often the common fungi found in our environment cause the fungal disease. Most of the fungi are not so very dangerous; however some can be very harmful for our health.

a) ***Aspergillus***

The most common pathogenic species are *Aspergillus fumigatus* and *A.flavus* they causes allergic disease. Some species of *Aspergillus* causes disease of grain crops, *e.g.* in crops of maize, where they synthesize toxins (mycotoxins including aflatoxin). The group of diseases which are caused by infection of *Aspergillus* are known as Aspergillosis.

Symptoms

The symptoms include fever, cough, fatigue pain in chest and bones or breathlessness, and cough. Usually, only patients with weakened immune

systems or with other lung infections are susceptible. Removing patients from high risk areas and the use of well fitted masks are two approaches that are commonly used as anti-fungal prophylaxis. Prevention of *Aspergillus* inhalation can be by nasal amphotericin B spray.

b) ***Cryptococcus***

Cryptococcus neoformans can cause intense form of meningitis and meningo-encephalitis in patients with HIV infection and AIDS. Those who are immune suppressed should avoid contact with birds, digging and dusty activities in areas heavily contaminated with bird droppings.

Symptoms

The symptoms of the infection depend on the parts of the body that are affected. The majority of symptoms of cryptococcosis occur in the lungs or the brain, or both. Cryptococcal infection may cause a pneumonia-like illness, with fever, coughing and shortness of breath. There may appear skin lesions as well. Another common form of cryptococcosis is central nervous system infection, such as meningoencephalitis. Symptoms may include fever, headache, or change in mental status.

c) ***Histoplasma***

Histoplasmacapsulatum can cause histoplasmosis in humans, dogs and cats. The fungus is most prevalent in the Americas, India and southeastern Asia. It is endemic in certain areas of the United States. Infection is usually due to inhaling contaminated air. Avoiding areas with bird and bat droppings may provide some protection. Wearing a respirator face mask can provide protection for workers in contaminated areas. Spraying soil with water before working the soil may help prevent release of spores into the air

Symptoms

Not everyone who inhales the fungal spores becomes sick. When illness occurs, the signs and symptoms appear in 3 to 17 days after exposure. The symptoms are similar to pneumonia and include fever, chills, sweats, a dry cough, malaise and chest pains. Joint pains can also be experience by some affected peoples. In absence of any treatment, the advancement of disease may develop weight loss, fatigue and shortness of breath in the affected people.

d) ***Pneumocystis***

Pneumocystis jirovecii (or *Pneumocystis carinii*) can cause a form of pneumonia in people with weakened immune systems, such as the elderly, premature children, and AIDS patients.

Symptoms

The symptoms of Pneumocystis include difficulty breathing, chest pain, chills,cough, fatigue (tiredness) and fever. In people with HIV/AIDS, Pneumocystis symptoms usually develop over several weeks and include a mild fever. In people with weakened immune systems (not due to HIV/AIDS), PCP symptoms usually develop over a few days, usually with a high fever.

e) ***Stachybotrys***

Stachybotryschartarum or "black mold" can cause respiratory damage and severe headaches. It frequently occurs in houses in regions that are chronically damp.

Symptoms

Toxic black mold causes serious symptoms and health problems such as mental impairment, breathing problems, damage to internal organs and sometimes even death. The most common black mold symptoms and health effects are associated with a respiratory response. Chronic coughing and sneezing, irritation to the eyes, mucus membranes of the nose and throat, rashes, chronic fatigue and persistent headaches can all be symptomatic of black mold exposure or black mold poisoning. Often compounded by allergic reaction to the black mold spores, these symptoms can include nausea, vomiting, and bleeding in the lungs and nose

f) ***Coccidioides***

Coccidioides causes Coccidioidomycosis also known as San Joaquin Fever or Valley Fever. Inhalation of dust contaminated with *Coccidioides* spores introduces the fungus to the lungs.

Symptoms

Symptoms of Valley fever may appear between 1 and 3 weeks after a person breathes in the fungal spores. About 60% of all infected people (without immunosuppression) have no symptoms and do not seek medical care. About 30%-35% of people who develop symptoms have flu-like symptoms (fever, cough, malaise, and chills) that resolve over about two to six weeks without treatment. Some may develop additional symptoms such as shortness of breath, night sweats, headaches, sputum production, and joint and muscle pains (symptoms resembling pneumonia).

g) ***Candida***

Candidiasis is a common type of fungal infection caused by different species yeast in the *Candida* family. *Candida* yeast thrives on moist surfaces of the body and is a common cause of vaginal infections. It can also cause an infection of the mouth or throat, known as thrush.

Symptoms

A candidiasis infection of the skin appears as a clearly defined patch of red, itchy skin, often leaking fluid. Scabs and pustules may be seen around the edge of the rash. It will usually be found in areas such as the groin, the folds of the buttocks, between the breasts, toes, or fingers, and in the navel. It may be hard to see on people with darker skin. In oral thrush white patches can form on the tongue and gums. If the white patches are wiped away the tissue beneath may bleed. It may become difficult to eat and the corners of the mouth may crack.

Prevention and Management of Fungal Agents

To reduce or remove the risks, various steps are needed and it will depend upon the distinct biohazards but there are a many common actions that can be applied:

- Many biological agents are communicated via air, such as toxins of mouldy grain or exhaled bacteria. For those it is important to avoid the formation of aerosols and dusts, including when cleaning or during maintenance.
- Good housekeeping, use of relevant warning signs and hygienic working strategy are main elements of safe and healthy working conditions
- For survive or resist heat, many microorganisms have developed mechanisms of dehydration or radiation, for example by producing spores. Decontamination measures used for clothing, waste and equipment are most appropriate hygienic measures for the workers that should be included.

3.4.3 Viral Agents

Viruses are very small cellular microorganisms, each of them being 15 to 400 nm in diameter and mostly containing only a single kind of nucleic acid. They may induce various types of diseases in plants, humans, and animals. These strictly intracellular parasites also have cellular specificity (cell tropism). The Host organism is strongly responsible for their replication; viruses cannot be multiplied outside the host. Different kinds of food products that get contaminated as they are processed are commonly responsible for infecting people who eat them. Contamination may happen through:

- Contact with animal and human faeces, or water that has been contaminated with the faeces and is being used for irrigation and washing, etc.
- Contact with \hands or objects that have been soiled by faeces.
- Contact with contaminated water having vomit in it.
- Contact with the objects present in an environment where an infected person had previously been present.
- Aerosol that contains virus since it is produced by an infected person. The extent and incidence of contamination may vary between products.

a) Norovirus and Sapovirus

In year 2002, four genera of Caliciviridae were renamed means they are getting new names. One of them is e Norovirus, which was earlier known as Norwalklike viruses, and is small and round in structure; and another one is Sapovirus, which was earlier known as Sapporolike viruses. The viral genome of these two viruses contains single-stranded plus sense RNA. The clinical indication of a Norovirus infection, is comparatively very mild. The symptoms shown in this viruses are diarrhoea and vomiting, and in extreme cases, convulsion. If Norovirus is introduced in a population or community, it may then be followed by a further spread of the disease. This is caused by the highly-infectious nature of the virus, which may result in extensive secondary infections, and leads to 50% of the contacts. In today's time, this virus is at present known to be the root cause of almost all outbreaks of nonbacterial gastroenteritis. Saproviruses primarily affect children below the age of five. In Argentina, mixed infections with Noroviruses, Sapoviruses and astroviruses were recorded in the faeces of children affected by acute gastroenteritis.

b) Enterovirus

The enteroviruses present in human are pervasive, enterically transmitted viruses that give rise to a severe range of illnesses in childrens as well as infants. Choice of enteroviruses is employed because of their ability to rapidly multiply in the intestine.

c) Hepatovirus

Hepatitis A virus (HAV) is the sole member of the genus Hepatovirus belongs to the family Picornaviridae. HAV act as a single antigenic type; it has four human genotypes, out of which three genotypes naturally affect the other primates that can be discriminated like non-human primates and chimpanzee.

d) Hepatitis E virus

The virus is transmitted by the faecal-oral route with faecal contaminated drinking water being the usual vehicle. Direct contamination is rare. Its primary targets are young adults, between the age of 15 and 30, and the overall death rate is between 0.5 and 3.0%. The disease is usually mild, however, pregnant women suffer a high fatality rate from fulminant hepatic failure.

e) Astrovirus

Astroviruses are small, 28 nm diameter non-enveloped, single-stranded RNA viruses which constitute the only members of the family Astroviridae. Human astrovirus is a remarkable cause of acute diarrhea among children, result in the outbreaks of diarrhea and occasionally in hospitalization. Disease caused by Astrovirusis generally sensitive than that of the disease caused by rotaviruses. However, recurrent co-infection of astrovirus with caliciviruses and rotavirus in childhood, diarrhea complicates the epidemiology. Infections are more common in winter.

f) Rotavirus

Rotaviruses are segmented double- stranded RNA viruses which belong to the family Reoviridae; which may explain the presence of mixed infections and their genetic variability. Rotaviruses can live for weeks in recreational and potable water and for at least four hours in the hand of humans. Mostly rotaviruses are found in waste water and can be concentrated by shellfish; may be linked with infectious disease that is followed by seafood consumption. The viruses are not destroyed by or relatively resistant to commonly used hard surface disinfectants and best hygienic hand- wash agents. It may be transmitted by contaminated hands and surfaces, faecal-oral contact and possibly by and respiratory spread. Oral-faecal transmission is easily transmitted by deficient sanitary conditions. In group A, Human rotaviruses, are considered the main root cause of viral gastroenteritis in all infants and most of the young children.

g) Flavivirus

Flavivirus is an enveloped virus with so many minuscule protuberances having a size of 40-60 nm in diameter and it belongs to genus flavivirus. Their genome consists of positive-sense RNA, single-stranded and non-segmented. In flavivirus genus, many viruses are designated as B group

arboviruses. According to antigenic relationships, diverse antigenic groups have been recognized midst the flavoviruses: Tick-borne encephalitis virus group (TBE) or Yellow fever virus group or Dengue group etc. These arboviruses may be present all over the world. Their vectors are particularly known as gnats of the tick-borne encephalitis virus group.

h) Hantavirus

Hantavirus is a RNA-virus which belongs to the family Bunyaviridae and mainly found in droppings, urine and saliva of infected deer mice and many wild rodents. It is identified as etiologic agents of two humans diseases. They may also cause a serious but rare lung disease called Hantavirus pulmonary syndrome (HPS) and "hemorrhagic fever with renal syndrome" of varying severity which is a human disease known as European Hantaviruses.

i) Foot-and- Mouth Disease virus

The main agent which may be responsible for Foot-and- Mouth Disease (FMD) virus is a RNA virus that belongs to the genus Aphthovirus of family Picornaviridae. Foot and Mouth Disease is a zoonosis, which is a transmissible disease to humans, but it may cross the species barrier with more difficulty and with less effect.

j) Aichi Virus

The type species of a new genus, Kobuvirus, of the family Picornaviridae, was first recognized in 1989 as the cause of oyster-associated nonbacterial gastroenteritis in man Aichi strain is a new type of small round virus which may mostly produces diarrhea in patients having the age group of 15 to 34 years, almost 50 to 76% of them possess neutralizing antibody.

Management of Viral Agents

Most of the food borne viral outbreaks can be identified or traced to food which can be manually handled by an infected food handler, instead of industrially processed foods. The viral contamination of food can be identified anywhere in the process which is start from the farm to fork, but most of the viral infections caused by food can be traced back to infected persons who handle food that is not heated or otherwise that can be treated afterwards. Therefore, then emphasis should be on personal hygiene during their preparation. During food pre-processing, if viruses are present, then residual viral infectivity may be present after some industrial processes. There are many other methods of inactivating viruses except sufficient heating that exist within a food are relatively inconstant, but viruses present in water and on the exposed surfaces can also be inactivated with ultraviolet light or also with strong oxidizing agents. Therefore, it is important that adequate surveillance should be given to good manufacturing practice (GMP) and good agriculture practice (GAP) to avoid viruses introduction onto the raw material and also into the food-manufacturing environment, and move to HACCP to make sure adequate control over viruses that may be present during the manufacturing process.

1) Heighten awareness about the presence and spread of these viruses by food handlers.

2) Optimize and standardize methods for the detection of food borne viruses.

3) To emphasize on the consideration of viruses in all stages of production, processing, and distribution, firstly set up the food safety quality control and strictly apply different management systems (GHP, GMP, and HACCP) and inspection of hygiene is more frequent of food manufacturers.

3.5 ENDOTOXINS AND ENTEROTOXINS

Toxins are having diverse nature; it includes small molecules, peptides, lipopeptides, cyclic peptides, alkaloids, Carbamate alkaloids, organophosphates, proteins, etc. There are two kinds of toxins called as Endotoxins and Enterotoxins. The important difference between Enterotoxin and Endotoxin is that Enterotoxin is a poisonous substance which may be released into or produced in intestines of bacterial cells while Endotoxin is a poisonous substance which may be produced within the bacterial cell while.

3.5.1 Endotoxins

Endotoxin is a poisonous substance which may present inside a bacterial cell that may released when the disintegration of bacterial cell occurs. They are lipopolysaccharides which are detected in the outer membrane of gram-negative bacteria. The outer membrane is distinctive to gram-negative bacteria. Hence, endotoxins are always related with gram-negative bacteria. Some of the species of gram-negative bacteria such as Endotoxin is a poisonous substance which may present inside a bacterial cell that comes out when the disintegration of bacterial cell occurs *Escherichia coli, Salmonella, Shigella, Pseudomonas, Haemophilus influenza, Neisseria, Bordetella pertussis* and *Vibrio cholera* are known as endotoxin producers.

3.5.2 Enterotoxins

An enterotoxin is a protein exotoxin liberated or released by a microorganism that may target intestine. Enterotoxins may be produced in or released into the intestines. Enterotoxins belong to the Exotoxin category. They are proteins as well as act as enzymes. Enterotoxins are those which may help in pore-forming toxins. Hence, they can creating pores in the epithelial cells of the intestine wall. The enterotoxins can increase the permeability by chloride ions in the cells of intestinal mucosa, it leads to secretory diarrhea. *E. coli* and *Staphylococcus aureus* are the two bacterial species which can generate such conditions by the enterotoxins.

Activities

- Identify the poisonous substances produced by plants, animals and microorganism which can induce harmful effect when exposed to tissues.
- Identify the food borne diseases by the organism which are toxic to human and animals.
- Find out the toxicity rate of the nearby areas where you are residing.
- Find out the toxicity level of the plant, animal and microorganism of your surroundings.

Check Your Progress 2

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) What are bacterial agents and viral agents?

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2) Write the methods for prevention of fungal agents.

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3) What are endotoxins and enterotoxins?

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3.6 LET US SUM UP

Biological Toxicants are chemicals that are toxin produced by living things in nature. Different type of living things such as bacteria in the animal gut, various plants which may produce potent toxins such as spore-forming bacteria producing anthrax toxin, ricin, red algae producing saxitoxin, certain insects and animals that may produce toxic venom, etc.; the list is very long. A terrorist or a nation state can potentially harvest and purify a toxin to be used against an enemy, perhaps by poisoning a water supply or by releasing a dust/aerosol in an airplane passenger compartment or inserting a fine powder in a letter. However, most poisonings occur accidentally when people eat contaminated food. Food intoxication is a type of food-borne illness which may be caused by ingesting the exotoxins made by different types of organisms such as bacteria, fungi etc or by consuming/taking the food items that are naturally toxic to humans and animals. Food intoxication leads to onset of illness which is usually very rapid and people feel sick. Every year around 300,000 peoples are reported to be hospitalizations and the fatality is almost 2000-4000.

Microbial food poisonings / food borne illnesses mainly fall under the one of the two categories that is known as Food Poisoning Infection vs. Food Poisoning Intoxication. The first is food infection, where the microorganism itself grows inside body. The second is food intoxication, where a chemical or natural toxin. Most bacterial (microbial) food poisonings are mainly food infections. Signs

and Symptoms may start within hours after eating the contaminated food, or they may begin days or even weeks later. Sickness caused by food poisoning generally lasts from a few hours to several days. The steps required to remove or reduce the major risks will be contigent upon the specific biohazard, but there are many common actions that may be tried or applied like various biological agents may be communicated through air, such as exhaled toxins of mouldy grain or bacteria. For those it is important to avoid the formation of aerosols and dusts, including when cleaning or during maintenance. Secondly, use of relevant warning signs, good housekeeping and hygienic working strategy are major key components of safe and the healthy working conditions. In some instances preventive measures can include vaccination that can be given to workers on a voluntary basis.

3.7 KEY WORDS

Endotoxin: It is a poisonous substance which may present inside a bacterial cell that may released when the disintegration of bacterial cell occurs. They are lipopolysaccharides which are detected in the outer membrane of gram-negative bacteria.

Rotaviruses: They are segmented double- stranded RNA viruses which belong to the family Reoviridae.

Mycotoxins: Mycotoxins are metabolites of fungus. Some of the mycotoxins are highly toxic to the animals and are potentially toxic even to human beings also.

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3.9 ANSWERS TO CHECK YOUR PROGRESS

Answers to Check Your Progress 1

Your answer should include the following points:

1) Biological toxicants are the poisonous substances which are released by the living organisms either for their defense like in the bees, ants, wasp or for predation like in the spiders, snakes, scorpion etc. Biological toxins are non–replicative, non-infectious material that can be hazardous in a very small quantity.

2) Biological toxicants are of various types depending upon the specific target they attack like Necrotoxins (cause tissue destruction via cell death), Neurotoxins (affect the nervous system), Haemotoxins (carried in the bloodstream and target RBCs), Cytotoxins, (kills individual cells). Biological Toxins can be classified according to their production source like, Cyanotoxins (produced by cyanobacteria), Mycotoxins (produced by fungi), Aitoxin (injected via the sting).

3) Food intoxication results when a person eats food containing toxins that cause illness. Toxins are produced by harmful microorganisms, the result of a chemical contamination, or are naturally part of a plant or seafood. Some bacteria, all viruses, and all parasites cause foodborne illness via infection. Food intoxicants can be classified as: (a) Naturally occurring toxicants (b) Toxicants from microorganisms.

Answers to Check Your Progress 2

Your answer should include the following points:

1) Bacteria are free-living organisms that are one celled, microscopic having size range from 1µm-10µm. Bacterial agents are defined by their ability to induce disease, incubation period, mode of transmission and case fatality rates. *Salmonellosis, Clostridium botulinum, Clostridium perfringers, Staphylococcus aureus* etc. are some of the examples of disease causing harmful bacterial agents.

   Viruses are small (15-400nm diameter), unicellular stern intracellular parasite which are not replicateoutside a particular living cell, viruses are not having its own metabolism as it is a inert particles. The more the time they survive in the environment which is infectious, the more the chance of transmission and to proliferate infection.Viral agents are the most known pathogens transmitted through food. Some of the viral agent causing various diseases: Enterovirus, Hepatovirus, Astrovirus, Flavivirus, Rotavirus, Aichi virus etc.

2) The steps required needed to remove or reduce the risks will depend upon the specific fungus and their mode of transmission and infection, however

there are a number of common act that can be tried or applied like hygienic working procedures, avoid the formation of aerosols, use of relevant warning signs and dusts, good housekeeping, and decontamination methods used for waste, appliances and clothing and use suitable hygienic measures.

3) Endotoxin is a substance which is poisonous in nature and may be present inside the cells of bacteria that comes out when the disintegration of bacterial cell occurs. Endotoxins are composed of lipopolysaccharides having high molecular weight which are detected in the outer membrane of the gram-negative bacteria. Endotoxins are substances which are heat stable and non-soluble in nature. Hence, it cannot be tear down by boiling.

   An enterotoxin is a protein exotoxin released by a microorganism that targets intestines. Enterotoxins are released or produced in or into intestines. Different types of bacterial species are capacity of producing enterotoxins. Enterotoxins belong to the Exotoxin category. They are proteins and can act as enzymes. Enterotoxins help in formation of pore-forming toxins.

UNIT 4 TOXICITY ASSESSMENT

Structure

4.0 INTRODUCTION

Toxicity may be expressed in a variety of forms depending on the chemical which is involved, the nature of the population exposed, and the conditions of exposure. We can define toxicity as the ability of a substance to cause adverse effects in living organisms. This ability to cause adverse effect is dependent upon several conditions. For example, it will depend on the quantity or the dose of the substance and whether the effects of the chemical are toxic, nontoxic or beneficial. In addition to dose, other factors may also influence the toxicity of the compound such as the route of entry, duration and frequency of exposure, variations between different species (interspecies) and variations among members of the same species (intraspecies). The potential hazards to humans may be acute, subchronic and chronic. The more specific types of toxicity that are determined include carcinogenicity; teratogenicity and reproductive toxicity, mutagenicity and neurotoxicity.

Toxicity testing is necessary to provide some basis for the regulation of substances those humans and other living things come into contact. It is used to determine the safety of range of chemicals used for example cosmetics, pharmaceuticals, food additives, pesticides, chemicals, additives and consumer products. A toxic effect can result from a natural or a manufactured substance and manifest a variety of symptoms which may be both immediate and long-term. Toxicity testing introduces a variety of methods and rates of exposure to the test organism, which

help in formulating a more accurate assessment of the risk of harm that the test substance may pose to human health and the environment. Toxicity assessment is characterization of the toxicological properties and effects of a chemical, with special emphasis on establishment of dose-response characteristics. This unit focuses on toxicity assessment and role of toxicity assessment in toxicological studies.

4.1 OBJECTIVES

After completing this unit, you will be able to:

- understand toxicity assessment and the routes of exposure,
- describe the dose response relationship,
- explain the lethal dose and lethal concentration, and
- discuss the various methods for toxicity assessment.

4.2 OVERVIEW OF TOXICITY ASSESSMENT

The term toxicity refers to the inherent potential of a substance to cause systemic damage to living organisms. All substances are toxic in quantity. Many therapeutic medications are acutely toxic, but beneficial when used at the appropriate level. Vitamin D, table salt, oxygen, and water are toxic in quantity. Thus, the mere presence of a substance does not automatically imply harm. This is why toxicity assessment is concerned with the type and degree of harm caused by differing amounts of a substance. A toxicity assessment is a tool to investigate the potential for a substance to cause harm and how much causes what kind of harm. Toxicity assessment is a major component of risk assessment.

A toxicity assessment provides information on how much of a chemical causes what kind of harm. If the toxicity assessment is based on an animal study, the degree of harm to humans must be extrapolated using mathematical models based on a variety of assumptions. Thus the toxicity assessment provides only an estimate of the harm to humans. As more toxicity studies on a particular chemical are conducted-dose-response studies on different species of animals for example, or epidemiological and *in vitro* (test tube) studies-scientists become more confident in their characterization of the toxicity of the substance. The risk assessor's job is to determine the real world risk to humans of a substance by combining information on toxicity and exposure. This job is made more complicated if data are collected from many different studies, but the results will be more likely to reflect the best estimates scientists can make.

4.3 ROUTES OF EXPOSURE

There are many routes by which a substance can enter the body. The major routes are discussed below:

1) **Inhalation:** The major route of entry for most chemicals in the form of vapors, gases, mists, or particulates is inhalation. Once inhaled, chemicals are either exhaled or deposited in the respiratory tract. The deposited chemical can, damage through direct contact with tissue or the chemical may diffuse into the blood through the lung-blood interface.

2) **Absorption**: The absorption of the chemical may be either through skin (dermal) or through eye. Dermal contact can cause redness or mild dermatitis or more severe effects like destruction of skin tissue or other debilitating conditions. Many chemicals can also cross the skin barrier and be absorbed into the blood system. Once absorbed, they may produce systemic damage to internal organs.

3) **Transformation:** Many chemicals are metabolized or transformed via chemical reactions in the body. In some cases, chemicals are distributed and stored in specific organs.

4) **Ingestion**: Chemicals that inadvertently get into the mouth and are swallowed do not generally harm the gastrointestinal tract itself unless they are irritating or corrosive. Chemicals that are insoluble in the fluids of the gastrointestinal tract (stomach, small, and large intestines) are generally excreted. Others that are soluble are absorbed through the lining of the gastrointestinal tract. They are then transported by the blood to internal organs where they can cause damage.

5) **Injection:** Substances may enter the body if the skin is penetrated or punctured by contaminated objects. Effects can then occur as the substance is circulated in the blood and deposited in the target organs.

6) **Storage:** Storage may reduce metabolism and therefore, increase the persistence of the chemicals in the body. The various excretory mechanisms (exhaled breath, perspiration, urine, feces, or detoxification) rid the body, over a period of time, of the chemical. For some chemicals elimination may be a matter of days or months; for others, the elimination rate is so low that they may persist in the body for a lifetime and cause deleterious effects.

4.4 TOXIC EFFECT

Toxic effect is defined as an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance." Such changes may be effected via acute, sub chronic or chronic exposure studies. Acute toxicity tests measure the immediate effects of exposure with an estimated time for peak effect of approximately eight hours after the initial exposure.

Toxicity tests usually focus on:

1) cytotoxicity (damages cells),
2) mutagenicity (alters genetic materials),
3) carcinogenicity (causes cancer)
4) teratogenicity (causes birth defects).

Toxic effects are classified as either acute or chronic.

4.4.1 Acute Effects

Acute effects happen very rapidly after a single exposure has occurred (food poisoning, breathing fumes from a chlorine spill). Sweating, nausea, paralysis, and death are examples of acute effects. In acute effect, there is a rapid and serious response to a high but short lived dose of toxic chemical. Acute poisons

interfere with essential physiological processes, leading to a variety of symptoms of distress and, if the interference is severe, to death.

Acute toxicity is relatively easy to measure. At high enough levels, the effects of toxins on bodily function are obvious and fairly consistent across individuals and species. These levels vary enormously for different chemicals. Almost everything is toxic at some level, and the difference between toxic and nontoxic chemicals is a matter of degree. The most widely used index of acute toxicity is LD50, the lethal dose for 50% of a population. This number is obtained by graphing the number of deaths among a 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. The dose is generally expressed as the weight of the chemical per kilogram of body weight, on the assumption that toxicity scales inversely with the size animal.

4.4.2 Chronic Effect

Chronic effects happen only after repeated long-term exposure (cigarette smoking, eating foods with low levels of contaminants, breathing polluted air). Cancer, organ damage, reproductive difficulties, and nervous system impairment are examples of chronic effects. In a chronic effect, in which the dose is relatively low but prolonged, and a time lag occurs between initial exposure and the full manifestation of the effect. Chronic toxins have more subtle effects, often setting in motion a chain of biochemical events that leads to disease states, including cancer.

Chronic effects are much more difficult to evaluate, especially at the low exposure levels that are likely to be encountered in the environment. In an experimental setting, the lower the dose, the fewer the animals that show any particular effect. To obtain statistically significant results, a study might have to include a prohibitively large number of animals. The only available recourse is to evaluate effects of a series of high doses, and then to extrapolate the dose response curve to the expected incidence at low doses. But extrapolation may have to extend over several orders of magnitude, and there is no assurance that the actual dose response function is linear. The biochemical mechanisms that control effects may be different at high and low doses. The controversy over this issue is especially heated in the context of animal testing for cancer.

Chronic effects fall into two categories: carcinogenic effects and non-carcinogenic effects.

Examples of non-carcinogenic chronic effects:

- **Organ damage:** Cirrhosis of the liver from long-term alcohol consumption; emphysema from long-term tobacco smoking.
- **Reproductive difficulty:** Decreased fertility from the pesticide DBCP (dibromochloropropane).
- **Nervous system impairment:** Mental retardation in people exposed to high levels of lead during early childhood

Check Your Progress 1

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) What do you understand by toxicity assessment?

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2) Enlist the different routes of exposure of toxic chemicals in the body.

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4.5 THE DOSE RESPONSE ASSESSMENT

The amount of a chemical that an organism (such as a person) is exposed to is called the dose, and the severity of the effect of that exposure is called the response. A dose response assessment is a scientific study to determine the relationship between dose and response, and how much dose is correlated with how much response. Response can be expressed as measured or observed incidence, percent response in groups of subjects (or populations), or as the probability of occurrence within a population. The process of characterizing the relation between the dose of an agent administered or received and the incidence of an adverse health effect in exposed populations and estimating the incidence of the effect as a function of human exposure to the agent. It takes into account the intensity of exposure, age pattern of exposure, and possibly other variables that might affect response, such as sex, lifestyle, and other modifying factors. A dose response assessment usually requires extrapolation from high to low dose and from animals to humans.

The second step in the assessment of risks to humans from potentially toxic agents, in which the relationship between the dose levels to which animals or humans are exposed and the health effect responses at each dose level are characterized quantitatively. Often the close response assessment is based on high dose experimental animal studies and applied to humans who are exposed at much lower doses. The process of characterizing the relation between the dose of an agent administered or received and the incidence of an adverse health effect in exposed populations and estimating the incidence of the effect as a function of human exposure to the agent.

4.6 DOSE-RESPONSE CURVE

Dose response curve is a mathematical relationship between the dose administered or received and the incidence of adverse health effects in the exposed population; toxicity values are derived from this relationship. The characteristics of exposure to a chemical and the spectrum of effects caused by the chemical come together in a correlative relationship that toxi-cologists call the dose response relationship.

This relationship is the most fundamental and pervasive concept in toxicology. To understand the poten-tial hazard of a specific chemical, toxicologists must know both the type of effect it produces and the amount, or dose, required to produce that effect. The relationship of dose to response can be illustrated as a graph called a dose response curve. There are two types of dose-response curves: one that describes the graded responses of an individual to varying doses of the chemi-cal and one that describes the distribution of responses to different doses in a population of individuals. The dose is represented on the x-axis and response on the y axis. An important aspect of dose-response relationships is the concept of threshold.

For most types of toxic responses, there is a dose, called a threshold, below which there are no adverse effects from exposure to the chemical. The human body has defenses against many toxic agents. Cells in human organs, especially in the liver and kidneys, break down chemicals into nontoxic substances that can be eliminated from the body in urine and feces. In this way, the human body can take some toxic insult (at a dose that is below the threshold) and still remain healthy. The identification of the threshold beyond which the human body cannot remain healthy depends on the type of response that is measured and can vary depending on the individual being tested. Thresholds based on acute responses, such as death, are more easily determined, while thresholds for chemicals that cause cancer or other chronic responses are harder to deter-mine. Even so, it is important for toxicologists to identify a level of exposure to a chemical at which there is no effect and to determine thresholds when possible.

The horizontal axis indicates the dose in mg/kg of body weight, while the vertical axis is the percent of maximum response. For a very low dose there is no or little response. The response increases with the dose until the maximum response is reached and increasing the dose has no additional effect.

4.7 LD_{50} AND LC_{50}

Lethal dose (50) or LD (50) is standard measure of the toxicity of a material that will kill half of the sample population of a specific test animal in a specified period through exposure via ingestion, skin contact, or injection. LD50 is measured in micrograms (or milligrams) of the material per kilogram of the test-animal's body weight; lower the amount, more toxic the material. Used in comparison of toxicities, LD50 values cannot be directly extrapolated from one species to the other or to humans. Also called median lethal dose, LD_{50} (originally abbreviated as DL50 for *dosis letalis*, 50%) is a test used in animal experiments. It was designed by the British pharmacologist J W Trevan in 1927. In other words LD_{50} is the dose of any substance tested required to kill half the number (50%) of test animals. The test shows how much of a substance must be taken before it becomes deadly.

Example: A reported "rat oral LD50 of 50 mg/kg" means that half of the rats that ingested a dose of 50 milligrams of the substance per kilogram of body weight died within 14 days.

The LD/50 test is used to determine the acute toxicity of a substance. This is the dose at which the test substance is lethal to 50% of the test animals. During the test period the animal forcibly inhales, ingests or is otherwise exposed to the

substance. Often the animals involved experience acute distress including "pain, convulsions, discharge, diarrhea and bleeding from the eyes and mouth."

LC stands for **"Lethal Concentration**". LC values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water. According to the OECD (Organization for Economic Cooperation and Development) Guidelines for the Testing of Chemicals, a traditional experiment involves groups of animals exposed to a concentration (or series of concentrations) for a set period of time (usually 4 hours). The animals are clinically observed for up to 14 days. The concentration of the chemical in air that kills 50% of the test animals during the observation period is the LC_{50} value. Other durations of exposure (versus the traditional 4 hours) may apply depending on specific laws.

The LD50 and LC50 are specific cases of the generalized values LDn and LCn. The LDn is the dose of a toxicant lethal to *n*% of a test population. The LCn is the exposure concentration of a toxicant lethal to *n*% of a test population. Thus, the LD50 is the statistically derived single dose of a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions. Similarly, the LC50 is the statistically derived exposure concentration of a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions.

Another important value that may be derived from the relationship shown is the threshold dose or concentration, the minimum dose or concentration required to produce a detectable response in the test population. The threshold value can never be derived with absolute certainty and therefore the lowest observed effect level (LOEL) or the NOEL have normally been used instead of the threshold value in deriving regulatory standards. There is a move to replace these values by the benchmark dose (BMD). This is defined as the statistical lower confidence limit on the dose that produces a defined response (called the benchmark response or BMR, usually 5 or 10%) in a given population under defined conditions for an adverse effect compared to background, defined as 0%.

In assessing the significance of LD50 or other toxicological values, it is necessary to note the units used in expressing dosage. Normally dosage is expressed in mg kg^{-1} body weight, but it may be expressed as mg cm^{-2} body surface area as this has been shown in a number of cases to permit more accurate extrapolation between animals of different sizes and from test mammalian species to humans. For biocides, selective toxicity is the key property, since they are to be used to kill pests with minimal harm to other organisms. Selective toxicity depends upon differences in biological characteristics that may be either quantitative or qualitative. Minimizing the amount of pesticide used and targeting its application is crucial to avoid harm to non-target organisms. On a body weight basis, it is assumed for toxicity data extrapolation that humans are usually about 10 times more sensitive than rodents. On a body surface area basis, humans usually show about the same sensitivity as test mammals, *i.e.* the same dose per unit of body surface area will give the same given defined effect, in about the same percentage of the population. Knowing the above relationships, it is possible to estimate the exposure to a chemical that humans should be able to tolerate. The toxicity rating can be different for different animals. Table 4.1 gives the toxicity classes given by Hodge and Sterner Scale.

Table 4.1: Toxicity Classes: Hodge and Sterner Scale

Routes of Administration					
		Oral LD_{50}	**Inhalation LC_{50}**	**Dermal LD_{50}**	**Probable**
Toxicity Rating	**Commonly Used Term**	**(single dose to rats) mg/kg**	**(exposure of rats for 4 hours) ppm**	**(single application to skin of rabbits) mg/kg**	**Lethal Dose for Man**
1	Extremely Toxic	1 or less	10 or less	5 or less	1 grain (a taste, a drop)
2	Highly Toxic	1-50	10-100	5-43	4 ml (1 tsp)
3	Moderately Toxic	50-500	100-1000	44-340	30 ml (1 fl. oz.)
4	Moderately Toxic	50-500	100-1000	44-340	30 ml (1 fl. oz.)
5	Practically Non-toxic	5000 – 15,000	10,000 – 100,000	2820 – 22,590	1 litre (or 1 quart)
6	15,000 or more	100,000	22,600 or more	1 litre (or 1 quart)	15,000 or more

Source: Nancy M. Trautmann, 2001

Table 4.2: Toxicity Categories Used for Human Poisons

Toxicity Category	**LD 50 (mg/kg)**	**Probable Lethal Dose for 70 kg Human Adult**	**Example Compounds**
Super toxic	<5	<0.35 g	Botulin Aflatoxin
Extremely toxic	5¨	0.35³.5 g	Cyanide Vitamin D (calciferol)
Very toxic	50@	3.5 g	Nicotine Caffeine
Moderately toxic	500µ,000	35è g	Aspirin (acetylsalicyclic acid) Salt (sodium chloride)
Slightly toxic	5,000±5,000	350±,050 g	Ethanol Trichloroethylene
Practicallynontoxic	>15,000	>1,050 g	Sugar (sucrose)

Source: Nancy M. Trautmann, 2001

The toxicity of a chemical depends on many factors, including whether it gets broken down, is stored in the body, or is excreted.

4.8 ASSESSING TOXICITY

All quantitative toxicity assessments are based on the dose-response concept. With the increase in the dose (exposure), the response (toxicity) also increases. The smaller the dose needed to cause an effect, the more potent (toxic) the substance is. For all compounds other than cancer-causing agents (carcinogens), it is assumed that there is a dose below which no effect occurs (a threshold). This is similar to a drug where too small of a dose has no beneficial effect. For carcinogens, it is often assumed that even the smallest dose can cause an effect. Although the dose response concept is used in all types of toxicity assessments, it is used somewhat differently for each of them**.** The toxicity assessment commonly involves two steps: hazard identification and dose response assessment. The outcome of a toxicity assessment is usually expressed as a toxicity value, such as a reference dose (RfD) or cancer slope factor (CSF), which incorporates the findings of the hazard and dose-response assessments and safety factors that address uncertainties in the assessment. A toxicity assessment may also conclude that a toxicity value cannot be developed because of inadequate data.

- **Acute toxic effects** are estimated by LD50 studies or observation of accidental exposures. Acute toxicity is assessed using observations of accidental human exposures or by conducting LD50 tests on experimental animals, usually rodents.

- **Chronic toxic effects** are estimated by dose-response studies on animals.

- **Carcinogenic effects** are estimated by a type of dose-response study called a carcinogenesis bioassay.

Toxicity Testing

Toxicity can be evaluated in whole organisms (*in vivo*) or using molecules or cells (*in vitro*). The main advantage of toxicity testing is that it detects toxic compounds based on their biological activity, and as such does not require a prior knowledge of the toxicant to identify its presence (unlike chemical analysis). Identification of the toxic component is required. Once a suspected toxicant is identified, modelling approaches (*in silico*) can be used to predict its toxicity based on the physico-chemical properties of the compound and its likely fate and transport in the environment.

Direct Toxicity Assessment

1) *In Vivo* Bioassays

Conventional toxicity testing relies on direct toxicity assessment in whole organisms (algae, shrimp, sea urchins, fish, rats, etc.).The organisms are exposed to the chemical(s) or mixture(s) of interest and monitored for any sign of adverse health effect. This can be either a gross morphological effect (such as weight loss, visible lesions, death) or more subtle biochemical markers, these being either biomarkers of exposure (an indicator of the internal dose, such as a metabolite in urine) or biomarkers of effect (an

indicator of a health effect, such as enzyme activity). The duration of the exposure depends on the type of toxicity detected or being monitored, from short-term acute effects (96 h or less), sub-acute (a couple of days), sub-chronic (a couple of weeks) to chronic effects (a significant portion of the organism's life expectancy). Depending on the species used, *in vivo* toxicity testing is generally seen as the most relevant predictor of human health effects. This is because *in vivo* tests include a measure of absorption, distribution, metabolism and excretion, all of which could modulate the toxicity of the sample.

2) *In Vitro* Bioassays (Bioanalytical Methods)

In vitro bioassays have been in use for drug discovery by the pharmaceutical industry for decades. In *in vitro* bioassays, molecules (e.g. enzymes) or whole cells are exposed to the chemical(s) or mixture(s) of interest and monitored for specific responses. However, for chemically-induced toxicity the initial interaction of the chemical at the molecular or cellular level is a necessary (but not sufficient) prerequisite for toxicity This is because toxicity occurs at the site of interaction of the toxicant (which can be either the parent compound or a metabolite) and the target biomolecule ("primary effect"). Organisms, however, have defense and detoxification mechanisms to cope with a certain degree of primary toxicity, and it is only when those defense mechanisms overcome, the observable toxicity occurs ("secondary effect"). This means that *in vitro* toxicity is likely to occur at significantly lower doses than *in vivo* effects but also means that a substance can be toxic *in vitro* but not *in vivo*. A variety of toxic effects can be monitored *in vitro*, from basal toxicity (cytotoxicity) and reactive toxicity (interaction with protein or DNA, which can then lead to carcinogenicity) that can potentially affect all cells, to specific toxicity that may only affect certain cells or organs (e.g. endocrine effects, neurotoxicity, immunotoxicity, liver toxicity, etc.). Typically, *in vitro* tests are carried out on specific cell types depending on the endpoint of interest. Some assays can be more variable than others, and thorough quality assurance / quality control procedures such as consistent use of positive and negative controls, monitoring of assay performance with control charts, quantification of detection limits, determination of reproducibility and robustness, use of inter-assay samples, intra- and inter-assay duplication and adoption of Good Laboratory Practices ensure the production of reliable high-quality data. Each type of bioassay has its advantages and limitations, and no single assay can provide a complete assessment of the biological activity of a sample. Therefore a battery of bioassays is required to rigorously assess the potential of a sample to cause biological effects in exposed organisms. *In vitro* assays are generally high-throughput short-term (<1 week) assays that provide a quick measurement of potential toxicity in a sample. These methods are presently at different stages of development and not all are presently suitable for inclusion in a monitoring program.

In Direct Toxicity Assessment

In silico approaches

Some of the shortcomings of *in vitro* bioassays, particularly the lack of integration of toxicokinetics, can be partly overcome by combining them with computer (*in silico*) modelling using structure-activity relationships (SAR). In SAR, the

chemical structure and other physico-chemical properties of the substance (once it is known) can be used to predict its toxicokinetics. *In silico* methods are very useful in the absence of other toxicological data, but are based on data from other chemicals and as such should be viewed with appropriate caution

4.8.1 Assessing Acute Toxicity

Acute Toxicity Tests

Acute toxicity standard tests though appears to be simple, routine methods but they should be performed strictly in accordance with standard requirements, observing recommended conditions and using indicated organisms. The tests are conducted either to determine the concentration of a test material (a chemical, or effluent) or the level of an agent (e.g., temperature or pH) that produces a deleterious effect on a group of test organisms during short-term exposure under controlled conditions. These tests are performed to determine the concentration of a particular chemical that would elicit a specific response or measurable endpoint from a test species in a relatively short period of time, usually from two to seven days. These tests are also applied in order to compare the sensitivity of different species or organisms at different stages of their development, to find the relationship with other variables that can affect overall results. They are also widely used for the assessment of the degree of potential harm to the environment caused by industrial discharges from the already existing or prospective factories or/and the application of new industrial processes. These tests must meet the following main requirements:

- They must be applicable to a wide range of chemicals
- They must be short, reproducible, sensitive, cheap and of low variability
- They must be standardized, accepted by the regulatory and scientific community.

4.8.2 Assessing Chronic Toxicity

Chronic Toxicity Tests

Chronic toxicity tests are more complex and the effects are studied for longer periods of time. The aim of these tests is to determine the concentration of a test material/ substance (e.g., a chemical, or effluent) that produces an adverse effect on a group of test organisms during long-term exposure under controlled conditions. Usually, chronic tests are conducted with a set of species of different phylogenetic level and different stage of development. Unlike acute toxicity tests, chronic toxicity studies evaluate not only mortality, but also investigate endpoints such as individual growth/growth rate, abnormal development, hatching time and success, reproduction (the total number of young individuals) and vitality of offspring, behaviour of individuals, physiological parameters, or histology. Using these methods, NOEC and LOEC can be calculated. NOEC (no observed effect concentration) is the highest concentration of the test substance that does not cause any observed and statistically significant adverse effects on the exposed organisms compared with controls. LOEC (the lowest observed effect concentration) is the lowest concentration of the substance used in a test that has statistically significant adverse effect on the exposed population of test organisms compared with controls. As legislation aims at preventing effects by reducing the amount of emitted chemicals, NOECs play a major role in applied ecotoxicology. Obtained results can be calculated to estimate MATC (Maximum

allowable toxic concentration), which is the geometric mean of LOEC and NOEC. Three categories of tests are commonly used to predict the chronic effects of toxic chemicals on aquatic organisms and data from these categories of tests can be used to estimate PNEC (Predicted no effect concentration). Chronic toxicity tests may be divided into: Full life cycle tests, i.e. those which evaluate the effects of a series of chemical concentrations on the reproduction, growth, survival and other parameters of one or more generations of a population of test organisms. Full life cycle tests are often applied to algae or invertebrate species which may be performed for more than one complete life cycle. However, they are not carried out on fish, as their performance requires long duration (till 1.2 years). Partial life cycle or sensitive life stage tests (embryolarvae of fish), i.e. those which evaluate survival, morphological, physiological parameters, hatching success, development abnormality etc. Duration of partial life cycle tests on fish can be as short as 7 days or as long as 60 days. However, the early life stage of fish (embryo/larval period) is the most sensitive period of fish life cycle. Therefore, embryo/larval tests are used for the more exact evaluation of substance toxicity to fish.

Chronic Toxicity **Can Be Divided Into Two Categories:**

- Cancer (carcinogenic toxicity).
- All other effects (non-carcinogenic toxicity).

Cancer is in a separate category because public concern about it is so great. People want to know if even one person in a million persons who are exposed to a substance will get cancer. To discover this, researchers conduct a specific type of dose-response study- a carcinogenesis bioassay. Non-carcinogenic effects are usually assessed with a different type of dose-response study.

Non-Carcinogenic Assessment

Non-carcinogenic chronic toxicity is assessed by studies to determine the smallest dose that causes any detectable effect. Scientists assess non-carcinogenic chronic toxicity by administering varying amounts of a substance (dose) to laboratory animals and noting the effects (responses), if any, at each dose. Essentially, the scientists look for the smallest dose that causes any detectable effect. This smallest dose is called the Lowest Observable Effect Level (LOEL).

Carcinogenesis Bioassay

Scientists assess carcinogenic toxicity very differently than they assess non-carcinogenic toxicity. This is in response to public fear about cancer. People want to know if even one in a million individuals will get cancer from exposure to a suspected carcinogen. To find this out with any degree of confidence by traditional dose-response studies, scientists would have to use several million test animals. The impracticality of such experiments has led to the development of the carcino-genesis bioassay. With a carcinogenesis bioassay, scientists are not looking for the safe level of exposure (NOEL). Rather, harm is assumed, and they are looking for the incidence, or risk, or harm. The carcinogenesis bioassay is a method of testing substances for carcinogenic effects that utilizes high-dose studies on laboratory animals to look for even the rare case of cancer. It is not necessarily the best scientific approach to assess the carcinogenic effects of chemicals. Instead it is a way to respond to public concerns by generating carcinogenic risk values with large margins of safety.

Example of A Carcinogenesis Bioassay: A carcinogenesis bioassay was performed for benzene on both rats and mice. Both sexes of each species got leukemia at the high doses administered. Extrapolating the cancer incidence at high dose to low dose and from rodents to humans resulted in the risk estimate that a benzene dose of 1 mg/kg/day will result in 3 cancers per 100 people exposed daily for a lifetime to that dose. This dose is much higher than anyone would be exposed to in the environment under normal conditions.

Check Your Progress 2

Note: a) Write your answer in about 50 words.

b) Check your progress with possible answers given at the end of the unit.

1) Discuss the dose response relationship with the help of dose response curve?

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2) What is LC?

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4.9 LET US SUM UP

A toxic effect can result from a natural or a manufactured substance and manifest a variety of symptoms which may be both immediate and long-term. Toxicity testing introduces a variety of methods and rates of exposure to the test organism, which help in formulating a more accurate assessment of the risk of harm that the test substance may pose to human health and the environment. Toxicity assessment is characterization of the toxicological properties and effects of a chemical, with special emphasis on establishment of dose-response characteristics. The guiding principles of toxicity testing is to check the effect of the test substances on laboratory animals and its direct toxic effect on human and secondly, the exposure of laboratory animals to high doses in order to evaluate its possible hazard on human that are exposed to much lower dose.

4.10 KEY WORDS

Cute : Occurring over a short time, usually a few minutes or hours.

Acute Exposure : One or a series of short term exposures generally lasting less than 24 hours

Administered Dose : The amount of a substance given to a test subject (human or animal) in determining dose-response relationships, especially through ingestion or inhalation.

Chronic : Occurring over a long period of time (more than 1 year).

Chronic Exposure : Long term exposure, usually lasting one year to a lifetime.

Dose Response : The relationship between the dose of a pollutant and the response (or effect) it produces on a biological system.

Dose Response Assessment : The amount of a chemical that an organism (such as a person) is exposed to is called the dose, and the severity of the effect of that exposure is called the response. A dose response assessment is a scientific study to determine the relationship between dose and response, and how much dose is correlated with how much response

4.11 REFERENCES AND SUGGESTED FURTHER READINGS

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4.12 ANSWERS TO CHECK YOUR PROGRESS

Answers to Check Your Progress 1

Your answer should include the following points:

1) Toxic effect is defined as an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance." Such changes may be effected via acute, sub chronic or chronic exposure studies. Acute toxicity tests measure the immediate effects of exposure with an estimated time for peak effect of approximately eight hours after the initial exposure. Subchronic toxicity tests occur over a period of weeks, while chronic affects tests measuring long term exposure last several months.

2) Inhalation, Absorption, Transformation, Ingestion , Injection, Storage

Answers to Check Your Progress 2

Your answer should include the following points:

1) Dose response curve is a mathematical relationship between the dose administered or received and the incidence of adverse health effects in the exposed population; toxicity values are derived from this relationship.

2) LC stands for **"Lethal Concentration**". LC values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water. According to the OECD (Organization for Economic Cooperation and Development) Guidelines for the Testing of Chemicals, a traditional experiment involves groups of animals exposed to a concentration (or series of concentrations) for a set period of time (usually 4 hours). The animals are clinically observed for up to 14 day.