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

The most important objectives of this course are:

Students will be able to:

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

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

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

4-Know the fundamental problems of toxicants in the world

5-Determine sources of toxicants

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

Toxicology: Theoretical syllabus

1st week	-introduction to toxicology -definition and scope -relationship to other sciences - sources of toxic compounds - movement of toxicants in the environment
2nd week	-Dose–response relationships -absorption and distribution -biotransformation and excretion
3rd week	- Classes of toxicant - toxicants in air -types of air toxicants - sources of air toxicants - Examples of air toxicant -environmental effects of air toxicants
4th week	Water and soil toxicants Sources of water and soil toxicants Examples of toxicant, lead, arsenic, cadmium, dioxins
5th week	Mercury, pesticides, nitrates and phosphates Oils and petroleum

6th week	Occupational toxicants Regulation of exposure levels Routes of exposure
7th week	Examples of industrial toxicants
8th week	Toxic action Mechanisms of acute toxicity
9th week	Mechanisms of acute toxicity
10th week	Organ toxicity, hepatotoxicity, mechanisms of hepatotoxicity Examples of hepatotoxicants carbon tetrachloride, ethanol
11th week	Neurotoxicity
12th week	Nephrotoxicity, example metal (cadmium), antimicrobial agents(cephalosporin)
13th week	Reproductive system toxicity
14th week	Endocrine toxicology
14th week	Respiratory tract toxicology
15th week	Immune system toxicology

LECT. 1

TOXICOLOGY

Learning Objectives
At the end of this chapter the student will be able to:

1. Find out about introduction, diffintion and scope.
2. Define different terminologies used in toxicology.
3. See the relationship to other sciences.
4. Understand the sources of toxic compounds
5. Understand movement of toxicants in the environment

INTRODUCTION

Definition and Scope

Toxicology can be defined as that branch of science that deals with poisons, and a poison can be defined as any substance that causes a harmful effect when administered، either by accident or design, to a living organism.

Toxicology also includes the study of harmful effects caused by physical phenomena, such as radiation of various kinds and noise.

Poison is a quantitative concept, almost any substance being harmful at some doses but, at the same time, being without harmful effect at some lower dose.

Vinyl chloride may be taken as an example. It is a potent hepatotoxicant at high doses, a carcinogen with a long latent period at lower doses, and apparently without effect at very low doses. Clinical drugs are even more poignant examples because, although therapeutic and highly beneficial at some doses، they are not without deleterious side effects and may be lethal at higher doses. Aspiri(acetylsalicylic acid), for example, is a relatively safe drug at recommended doses and is taken by millions of people worldwide. At the same time, chronic use can cause deleterious effects on the gastric mucosa, and it is fatal at a dose of about 0.2 to 0.5g/kg. Approximately 15% of reported accidental deaths from poisoning in children result from ingestion of salicylates, particularly aspirin.

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

The measurement of toxicity is also complex. Toxicity may be acute or chronic, and may vary from one organ to another as well as with age, genetics, gender, diet, physiological condition, or the health status of the organism. As opposed to experimental animals, which are highly inbred, genetic variation is a most important factor in human toxicity since the human population is highly outbred and shows extensive genetic variation. Even the simplest measure of toxicity, the LD50 (the dose required to kill 50% of a population under stated conditions) is highly dependent on the extent to which the above variables are controlled. LD50 values, as a result, vary markedly from one laboratory to another.

Exposure of humans and other organisms to toxicants may result from many activities: intentional ingestion, occupational exposure, environmental exposure, as well as accidental and intentional (suicidal or homicidal) poisoning. The toxicity of a particular compound may vary with the portal of entry into the body, whether through the alimentary canal, the lungs, or the skin. Experimental methods of administration such as injection may also give highly variable results; thus the toxicity from intravenous (IV), intraperitoneal (IP), intramuscular (IM), or subcutaneous (SC) injection of a given compound may be quite different. Toxicity may vary as much as tenfold with the route of administration. Following exposure there are multiple possible routes of metabolism, both detoxifying and activating, and multiple possible toxic endpoints (Figure 1).

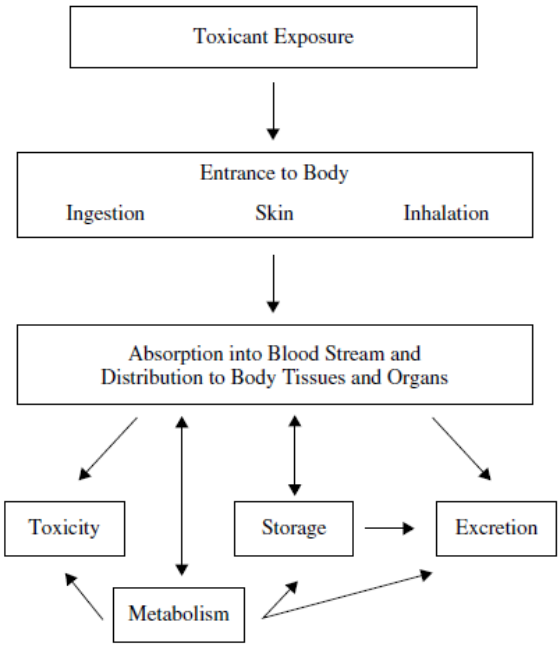


Figure 1. Fate and effect of toxicants in the body.

Relationship to Other Sciences

Toxicology is highly eclectic science and human activity drawing from, and contributing to, a broad spectrum of other sciences and human activities. At one end of the spectrum are those sciences that contribute their methods and philosophical concepts to serve the needs of toxicologists, either in research or in the application of toxicology to human affairs. At the other end of the spectrum are those sciences to which toxicology contributes.

In the first group chemistry, biochemistry, pathology, physiology, epidemiology، immunology, ecology, and biomathematics have long been important while molecular biology has, in the last two or three decades, contributed to dramatic advances in toxicology.

In the group of sciences to which toxicology contributes significantly are such aspects of medicine as forensic medicine, clinical toxicology, pharmacy and pharmacology، public health, and industrial hygiene. Toxicology also contributes in an important way to veterinary medicine, and to such aspects of agriculture as the development and safe use of agricultural chemicals. The contributions of toxicology to environmental studies has become increasingly important in recent years.

The use of dinitrophenol and other uncoupling agents to study oxidative phosphorylation and the use of α-amanitin to study RNA polymerases are but two of many examples. The field of toxicology has expanded enormously in recent decades, both in numbers of toxicologists and in accumulated knowledge. This expansion has brought a change from a primarily descriptive science to one which utilizes an extensive range of methodology to study the mechanisms involved in toxic events.

SOURCES OF TOXIC COMPOUNDS

Given the enormous number of toxicants, it is difficult to classify them chemically، either by function or by mode of action, since many of them would fall into several classes. Some are natural products, many are synthetic organic chemicals of use to society, while others are by-products of industrial processes and waste disposal. It is useful, however, to categorize them according to the expected routes of exposure oraccording to their uses.

a. Exposure Classes

Exposure classes include toxicants in food, air, water, and soil as well as toxicants characteristic of domestic and occupational Settings.

b. Use Classes

Use classes include drugs of abuse, therapeutic drugs, agricultural chemicals, food additives and contaminants, metals, solvents, combustion products, cosmetics, and toxins. Some of these, such as combustion products, are the products of use processes rather than being use classes.

MOVEMENT OF TOXICANTS IN THE ENVIRONMENT

Chemicals released into the environment rarely remain in the form, or at the location of release. For example, agricultural chemicals used as sprays may drift from the point of application as air contaminants or enter runoff water as water contaminants. Many of these chemicals are susceptible to fungal or bacterial degradation and are rapidly detoxified, frequently being broken down to products that can enter the carbon, nitrogen, and oxygen cycles. Other agricultural chemicals, particularly halogenated organic compounds, are recalcitrant to a greater or lesser degree to metabolism by microorganisms and persist in soil and water as contaminants; they may enter biologic food chains and move to higher trophic levels or persist in processed crops as food contaminants. This same scenario is applicable to any toxicant released into the environment for a specific use or as a result of industrial processes, combustion, and so on. Chemicals released into the environment are also susceptible to chemical degradation, a process often stimulated by ultraviolet light. Although most transport between inanimate phases of the environment results in wider dissemination, at the same time dilution of the toxicant in question and transfer among living creatures may result in increased concentration or bioaccumulation. Lipid soluble toxicants are readily taken up by organisms following exposure in air, water, or soil. Unless rapidly metabolized, they persist in the tissues long enough to be transferred to the next trophic level. At each

level the lipophilic toxicant tends to be retained while the bulk of the food is digested, utilized, and excreted, thus increasing the toxicant concentration. Thus the eggshell thinning in certain raptorial birds was almost certainly due to the uptake of DDT and DDE and their particular susceptibility to this type of toxicity. Simplified food chains are shown in Figure 2.

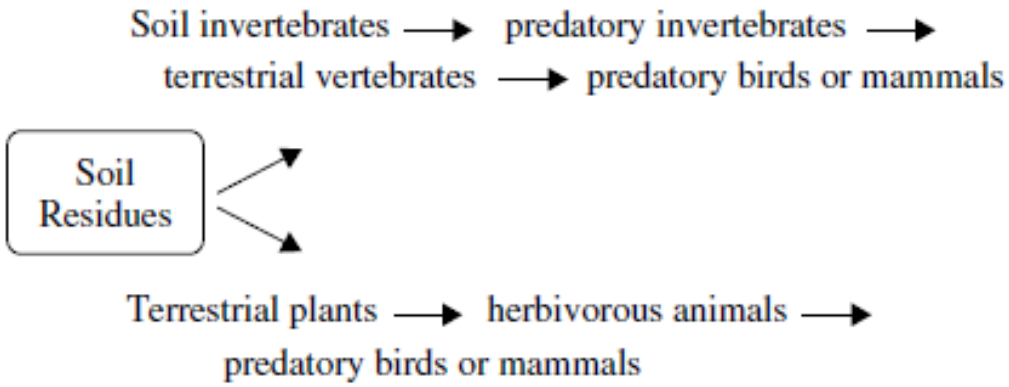


Figure 2. Examples of simplified food chains.

LECT. 2

TOXICOKINETICS AND TOXICODYNAMICS

Learning Objectives
At the end of this chapter the student will be able to:

1. Define Toxicokinetics and Toxicodynamics.
2. Understanding the relationship of the body with the poisons
3. Understanding the dose response relationship

Toxicokinetics deals with absorption, distribution، biotransformation (biotransformation) and excretion of chemicals(fig. 3)

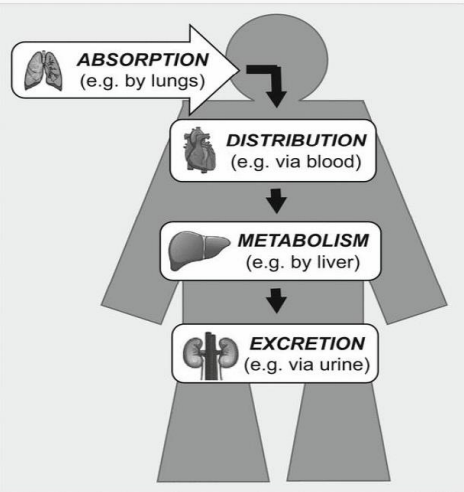


Figure 3. toxokinetics

Toxicodynamics deals with the biochemical and physiological effects of chemicals to the body and mechanisms of their actions.

A. Toxicokinetics

i) Absorption

Absorption is the process by which the chemical enters the body. It depends on the route of administration, dissociation (to become ionized), dissolution (ability of solid dosage form to become soluble), concentration, blood flow to the site, and the area of the absorptive site.

The common sites of absorption (routes of exposure) are

- ❖ Oral route – the GIT is the most important route of absorption, as most acute poisonings involve ingestions.
- ❖ Dermal route – lipid solubility of a substance is an important factor affecting the degree of absorption through the skin.
- ❖ Inhalational route – toxic fumes, particulate and noxious gases may be absorbed through the lungs.

Bioavailability is the fraction of unchanged drug reaching the systemic circulation following of non-vascular administration. Therefore, a portion of the chemical fails to reach the systemic circulation in original form after oral administration.

ii) Distribution

Distribution-is defined as the apparent volume into which a substance is distributed.(fig. 4)

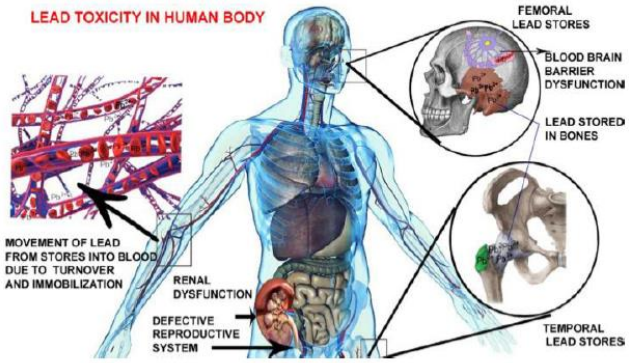


Figure 4. Distribution

Volume of distribution (Vd) is calculated from the dose taken and the resulting plasma concentration:

Vd = dose /plasma concentration

The importance of volume of distribution in toxicology is

1-Predicting peak blood concentration of the chemical taken

2-Calculating the amount of substance in the body to verify the quantity ingested

3-Deciding whether to apply systemic toxin elimination techniques

- Factors determining the rate of distribution of chemicals in the body are

1 -Protein binding – chemicals highly bound to protein have small volume of distribution

2 -Plasma concentration – when the volume of distribution of chemicals is small, most of the chemical remains in the plasma

3-Physiological barriers – chemicals will not uniformly distributed to the body due to specialized barriers e .g blood brain barrier

4 -Affinity of chemicals to certain tissues – the concentration of a chemical in certain tissues after a single dose may persist even when its plasma concentration is reduced e .g Lead concentrate in bone tissue

E .g A 60Kg epileptic victim attempted suicide by ingesting Phenytoin tablets. Vd listed is 0.6 L/Kg. Peak blood concentration measured by the laboratory is 50mg/ L. What is the dose of the drug that was taken by the victim؟

Dose=plasma concentration x Vd

=50mg/L x (0.6L/Kg x 60Kg)

=1800mg

iii) Biotransformation (metabolism)

Biotransformation is the biochemical transformation of a chemical. It is a process by which the body transforms a chemical and makes it more water soluble so the chemical can be eliminated more rapidly via the kidney into the urine. Biotransformation can produce metabolites that are pharmacologically active and toxic(fig. 5)

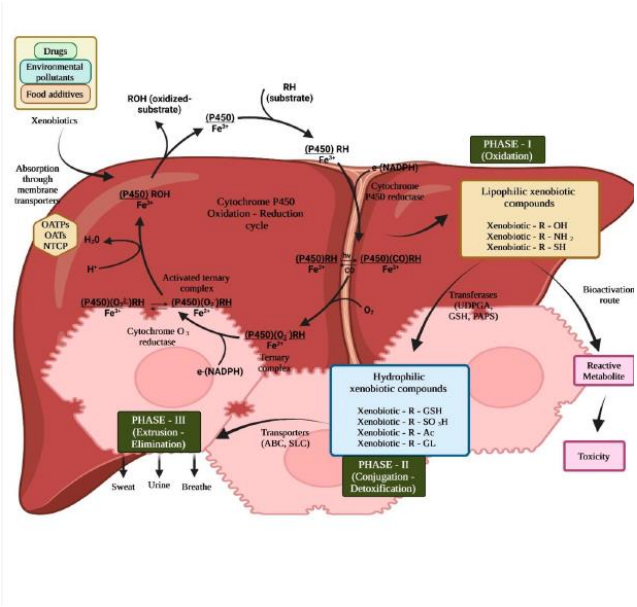


Fig. 5. Metabolism

E.g. parathion→ parathoxon

(toxic metabolite). Liver is the major site of biotransformation for many chemicals & other organs that are involved are lungs، kidneys, skin &so on. Interactions during biotransformation includes

There are two phases of biotransformation

Phase I – the drug is converted into more polar compound e .g oxidation, reduction, &hydrolysis

Phase II (conjugation) – a drug or its metabolite is conjugated with an endogenous substance e .g glucuronide conjugate

Enzyme inhibition- by this the biotransformation of drugs is delayed & is a cause of increased toxicity

Enzyme induction- by this the biotransformation of drugs is

accelerated & is a cause of therapeutic failure

First – pass effect – is the biotransformation of some chemicals by the liver during the initial pass from the portal circulation after oral administration.

Half life (t ½) –is the time required to reduce the blood concentration of the chemical to half.

IV) Excretion

Excretion is the final means of chemical elimination, either as metabolites or unchanged parent chemical(fig. 6).

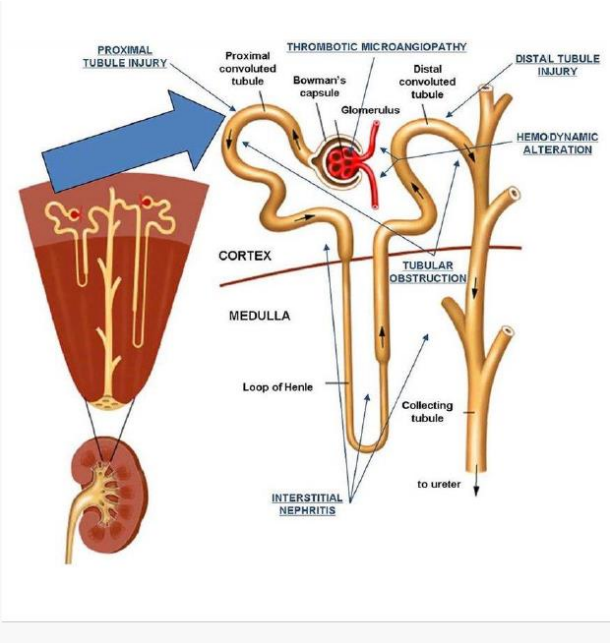


Figure 6. Execretion

Excretion through the lungs is the major route for gaseous substances; and in the case of non-volatile water – soluble drugs, the kidneys are the most important routes of excretion. Additional routes include sweat, saliva, tears, nasal secretions, milk, bile and feces. Clearance – elimination of chemicals from the body may be described by the term clearance (CL). It is a quantitative measure of the volume of blood cleared of drug per unit time, usually expressed in milliliter pe4r minute.

Clearance is calculated as follows

$$\mathbf{CL = 0.7\ (VD)/\ (t1/2) = ml/min}$$

Where the VD is expressed in milliliter per kilogram & the half-life is expressed in minutes of hours.

Toxicodynamics

Toxicodynamics is the mechanism of action of a toxic chemical to the body (what chemicals do to the body). The targets for the toxicodynamic actions of toxic chemicals are

1. Enzymes
2. Membrane receptors
3. Intracellular receptors
4. Ion channel

The general dose-response principles are of crucial importance in determining the severity of the intoxication. We have two types of responses so called Quantal dose response (all- or– none response) & graded dose response (when dose increases, the response increases in graded fashion). Both responses show a typical dose response relation.

The parameters that are derived from the dose response relationships are(fig. 7)

Median lethal dose (LD50) – is the dose which is expected to kill %50of the population in the particular group.

Median effective dose (ED50) –is the dose that produces a desired response in 50% of the test population when pharmacological effects are plotted against dosage.

Median toxic dose (TD50) – is the dose which is expected to bring toxic effect in 50% of the population in the particular group.

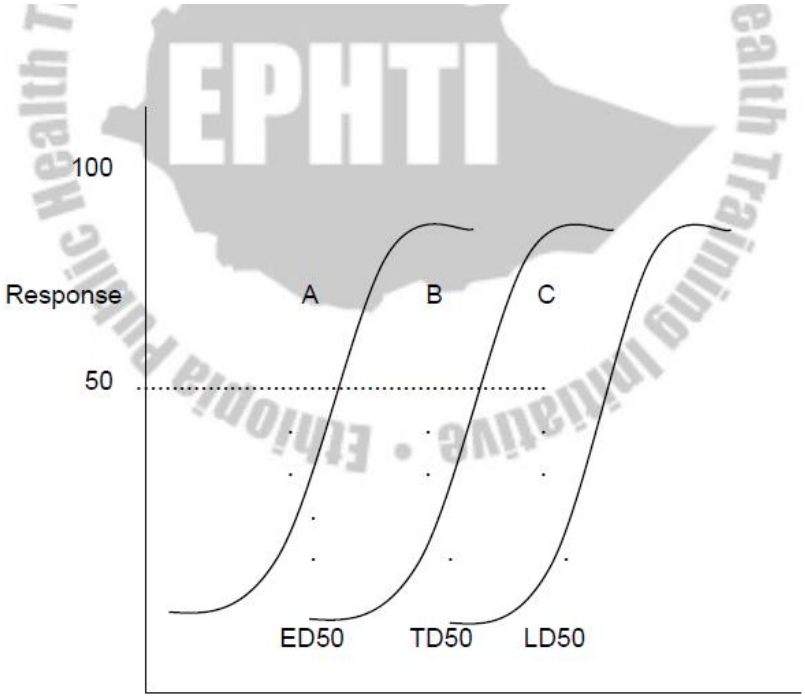


Figure 7. A typical dose-response curve.

LECT. 3

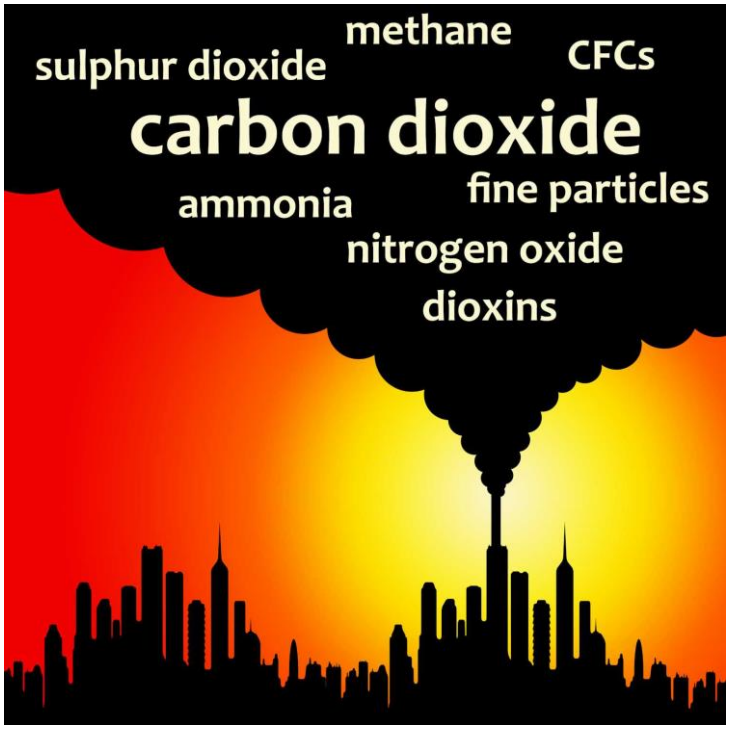
AIR, WATER AND SOIL POLLUTANTS

Learning Objectives

At the end of this chapter the student will be able to:

1. Describe Classes of toxicant
2. Describe toxicants in air
3. Knowing the types of air toxicants
4. Explain the sources of air toxicants
5. Illustrate the Examples of air toxicant
6. Understanding the environmental effects of air toxicants

AIR POLLUTANTS



Types of Air Pollutants

1. **Gaseous Pollutants.** These substances are gases at normal temperature and pressure as well as vapors evaporated from substances that are liquid or solid. Among pollutants of greatest concern are carbon monoxide (CO), hydrocarbons, hydrogen sulfide (H2S), nitrogen oxides (NxOy), ozone (O3).
2. **Particulate Pollutants.** Fine solids or liquid droplets can be suspended in air. Some of the different types of particulates are defined as follows:
   a. **Dust.** Relatively large particles about 100 μm in diameter that come directly from substances being used (e.g., coal dust, ash, sawdust, cement dust, grain dust)

b. **Fumes.** Suspended solids less than 1 μm in diameter usually released from metallurgicalor chemical processes, (e.g., zinc and lead oxide).
c. **Mist.** Liquid droplets suspended in air with a diameter less than 2.0 μm, (e.g; sulfuric acid mist).
d. **Smoke.** Solid particles (0.05–1.0 μm) resulting from incomplete combustion of fossil fuels.
e. **Aerosol.** Liquid or solid particles (<1.0 μm) suspended in air or in another gas.

Sources of Air Pollutants

- **Natural Pollutants**. Many pollutants are formed and emitted through natural processes. An erupting volcano emits particulate matter as well as gases such as sulfur dioxide, hydrogen sulfide, and methane; such clouds may remain airborne for long periods of time. Forest and prairie fires produce large quantities of pollutants in the form of smoke, unburned hydrocarbons, CO, nitrogen oxides, and ash. Plants also produce pollen and spores, which cause respiratory problems and allergic reactions.
- **Anthropogenic Pollutants**

  - These substances come primarily from three sources:

  (1) combustion sources that burn fossil fuel for heating and power, or exhaust emissions from transportation vehicles that use gasoline or diesel fuels;

  (2) industrial processes.

  (3) mining and drilling.

  The principal pollutants from combustion are fly ash, smoke, sulfur, and nitrogen oxides, as well as CO and CO2. Combustion of coal and oil, both of which contain significant amounts of sulfur, yields large quantities of sulfur oxides. One effect of the production of sulfur oxides is the

formation of acidic deposition, including **acid rain**. Nitrogen oxides are formed by thermal oxidation of atmospheric nitrogen at high temperatures; thus almost any combustion process will produce nitrogen oxides. Carbon

monoxide is a product of incomplete combustion; the more efficient the combustion, the higher is the ratio of CO2 to CO.

Transportation sources, particularly automobiles, are a major source of air pollution and include smoke, lead particles from tetraethyl lead additives, CO, nitrogen oxides, and hydrocarbons.

- **Indoor Pollutants.**

In general, the term "indoor air pollution" refers to home and nonfactory public buildings such as office buildings and hospitals. Pollution can come from heating and cooking, pesticides, tobacco smoking, radon, gases, and microbes from people and animals.

indoor air pollution is a particular problem in developing countries. Wood, crop residues, animal dung, and other forms of biomass are used extensively for cooking and heating-often in poorly ventilated rooms. For women and children, in particular, this leads to high exposures of air pollutants such as CO and polycyclic aromatic hydrocarbons.

Examples of Air Pollutants

1. **Carbon Monoxide.** Carbon monoxide combines readily wit hemoglobin (Hb) to form carboxyhemoglobin (COHb), thus preventing the transfer of oxygen to tissues. The affinity of hemoglobin for CO is approximately 210 times its affinity for oxygen. Concentrations of 100 ppm can cause headaches, dizziness, nausea, and breathing difficulties.
2. **Sulfur Oxides.** Sulfur dioxide is a common component of polluted air that results primarily from the industrial combustion of coal, with soft coal containing the highest levels of sulfur. The sulfur oxides tend to

adhere to air particles and enter the inner respiratory tract, where they are not effectively removed. In the respiratory tract, SO2 combines readily with water to form sulfurous acid, resulting in irritation of mucous membranes and bronchial constriction.

3. **Nitrogen Oxides.** Nitrogen dioxide (NO2), a gas found in photochemical smog, is also a pulmonary irritant and is known to lead to pulmonary edema and hemorrhage.
4. **Ozone.** A highly irritating and oxidizing gas is formed by photochemical action of ultraviolet (UV) light on nitrogen dioxide in smog. The resulting ozone can produce pulmonary congestion, edema, and hemorrhage.

$$NO_2 + \text{UV light} \longrightarrow NO + O^{\bullet}$$
$$O^{\bullet} + O_2 \longrightarrow O_3$$

5. **Lead.** One of the most familiar of the particulates in air pollutants is lead, with young children and fetuses being the most susceptible. Lead can impair renal function, interfere with the development of red blood cells, and impair the nervous system, leading to mental retardation and even blindness. The two most common routes of exposure to lead are inhalation and ingestion.
6. **Hydrocarbons (HCs) or Volatile Organic Compounds (VOCs).** Many gasoline pumps now have VOC recovery devices to reduce pollution.

Environmental Effects

A. **Vegetation.** Pollutants may visibly injure vegetation by bleaching, other color changes, and necrosis, or by more subtle changes such as alterations in growth or reproduction.

B. **Atmospheric Effects.** The presence of fine particles (0.1–1.0 mm in diameter) or NO2 in the atmosphere can result in atmospheric haze or reduced visibility due to light scattering by the particles. increase in CO2 levels would result in a global increase in air temperatures.

C. **Acidic Deposition.** Acidic deposition is the combined total of wet and dry deposition, with wet acidic deposition being commonly referred to as **acid rain**. Normal uncontaminated rain has a pH of about 5.6, but acid rain usually has a pH of less than 4.0. Much of the acidity in rain may be neutralized by dissolving minerals in the soil such as aluminum, calcium, magnesium, sodium, and potassium, which are leached from the soil into surface waters. The ability of the soil to neutralize or buffer the acid rain is very dependent on the alkalinity of the soil.

LECT. 4

WATER AND SOIL TOXICANTS

Learning Objectives

At the end of this chapter the student will be able to:

1. Ellustrate Introduction about Water and soil toxicants

2. Describe the Sources of water and soil toxicants

3. Mention the Examples of toxicant, lead, arsenic, cadmium, dioxins

INTRODUCTION

With three-quarters of the earth's surface covered by water and much of the remainder covered by soil, it is not surprising that water and soil serve as the ultimate sinks for most anthropogenic chemicals. Until recently the primary concern with water pollution was that of health effects due to pathogens, and in fact this is still the case in most developing countries. In the United States and

other developed countries, however, treatment methods have largely eliminated bacterial disease organisms from the water supply, and attention has been turned to chemical contaminants.

Sources of Water and Soil Pollutants

Surface water can be contaminated by **point** or **nonpoint sources**. An effluent pipe from an industrial plant or a sewage-treatment plant is an example of **a point source**; a field from which pesticides and fertilizers are carried by rainwater into a river is an example of **a nonpoint source**. Industrial wastes probably constitute the greatest single pollution problem in soil and water. These contaminants include organic wastes such as solvents, inorganic wastes, such as chromium and many unknown chemicals. Contamination of soil and water results when by-product chemicals are not properly disposed of or conserved. In addition industrial accidents may lead to severe local contamination. For a more in-depth discussion of sources and movements of water pollutants.

Contamination of soil and water also results from the use of pesticides and fertilizers. Persistent pesticides applied directly to the soil have the potential to move from the soil into the water and thus enter the food chain from both soil and water. In a similar way fertilizers leach out of the soil or runoff during rain events and flow into the natural water systems.

Examples of Pollutants

Metals that are of environmental concern fall into three classes:

(1) Metals that are suspected carcinogens

(2) Metals that move readily in soil, and

(3) Metals that move through the food chain.

Lead: The heavy metals of greatest concern for health with regard to drinking water exposure are lead and arsenic. The sources of lead in drinking water that are most important are from lead pipes and lead solder. Also of concern is the seepage of lead from soil contaminated with fallout from leaded gasoline and

seepage of lead from 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.

Arsenic: Drinking water is at risk for contamination by arsenic from the leaching of inorganic arsenic compounds formerly used in pesticide sprays, from the combustion of arsenic-containing fossil fuels, and from the leaching of mine tailings and smelter runoff. Chronic high-level exposures can cause abnormal skin pigmentation, hyperkeratosis, nasal congestion, and abdominal pain. At lower levels of chronic exposure, cancer is the major concern. Epidemologic studies have linked chronic arsenic exposure to various cancers, including skin, lungs, and lymph glands.

Cadmium: One of the most significant effects of metal pollution is that aquatic organisms can accumulate metals in their tissues, leading to increased concentrations in the food chain. Concern about long-term exposure to cadmium intensified after recognition of the disease Itai-Itai (painful-painful) in certain areas of Japan(fig. 8). The disease is a combination of severe kidney damage and painful bone and joint disease and occurs in areas where rice is contaminated with high levels of cadmium. This contamination resulted from irrigation of the soil with water containing cadmium released from industrial sources. Cadmium toxicity in Japan has also resulted from consumption of cadmium-contaminated fish taken from rivers near smelting plants.

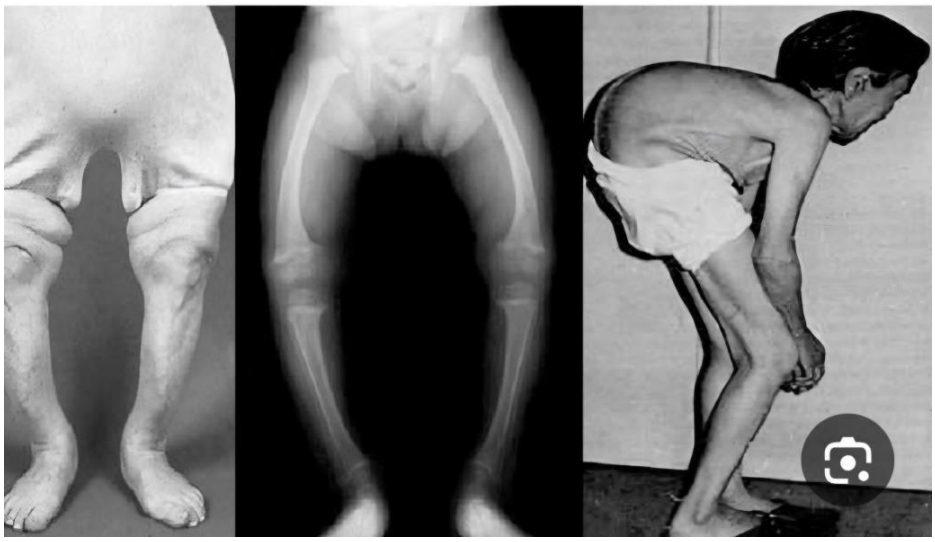


Figure 8. Tai Tai disease

LECT. 5

WATER AND SOIL TOXICANTS

Learning Objectives

At the end of this chapter the student will be able to:

- Mention the Examples of toxicants, Mercury, pesticides, nitrates and phosphates Oils and petroleum

Mercury: In Japan in the 1950s and 1060s, wastes from a chemical and plastics plant containing mercury were discharged into Minamata Bay. The mercury was converted to the readily absorbed methylmercury by bacteria in the aquatic sediments. Consumption of fish and shellfish by the local population resulted in numerous cases of mercury poisoning, or Minamata disease(fig. 9).

Figure 9. Minimata disease

Pesticides are also a major source of concern as water and soil pollutants. Because of their stability and persistence, the most hazardous pesticides are the organochlorine compounds such as DDT, aldrin, dieldrin, and chlordane. Persistent pesticides can accumulate in food chains; for example, shrimp and fish can concentrate some pesticides as much as 1000- to 10,000-fold. This bioaccumulation has been well documented with the pesticide DDT, which is now banned in many parts of the world. In contrast to the persistent insecticides, the organophosphorus (OP) pesticides, such as malathion, and the carbamates, such as carbaryl, are short-lived and generally persist for only a few weeks to a few months.

Nitrates and phosphates are two important nutrients that have been increasing markedly in natural waters since the mid-1960s. Sources of nitrate contamination include fertilizers, discharge from sewage treatment plants, and leachate from septic systems and manure. Nitrates from fertilizers leach readily from soils, and it has been estimated that up to 40% of applied nitrates enter water sources as runoff and leachate. Fertilizer phosphates, however, tend to be absorbed or bound to soil

particles, so that only 20% to 25% of applied nitrates are leached into water. Phosphate detergents are another source of phosphate, one that has received much media attention in recent years.

The increase in these nutrients, particularly phosphates, is of environmental concern because excess nutrients can lead to “algal blooms” or eutrophication, as it is known, in lakes, ponds, estuaries, and very slow moving rivers. The algal bloom reduces light penetration and restricts atmospheric reoxygenation of the water. When the dense algal growth dies, the subsequent biodegradation results in anaerobic conditions and the death of many aquatic organisms.

There are two potential adverse health effects from nitrates in drinking water:

1) nitrosamine formation and (2) methemoglobinemia. Ingested nitrates can be converted to nitrites by intestinal bacteria.

After entering the circulatory system, nitrite ions combine with hemoglobin to form methemoglobin, thus decreasing the oxygen-carrying capacity of the blood and resulting in anemia or blue-baby disease. It is particularly severe in young babies who consume water and milk-formula prepared with nitrate-rich water. Older children and adults are able to detoxify the methemoglobin as a result of the enzyme methemoglobin reductase, which reverses the formation of methemoglobin. In infants, however, the enzyme is not fully functional. Certain nitrosamines are known carcinogens.

Volatile organic compounds (VOCs): are other common groundwater contaminants. They include halogenated solvents and petroleum products, collectively referred to as VOCs. Both groups of compounds are used in large quantities by a variety of industries, such as degreasing, dry cleaning, paint, and the military. The EPA’s National

Priority List includes 11 VOCs: trichloroethylene, toluene, benzene, chloroform, tetrachloroethylene, 1,1,1-trichloroethane, ethylbenzene، trans-1,2-dichloroethane, xylene, dichloromethane, and vinyl chloride. The physical and chemical properties of VOCs permit them to move rapidly into groundwater, and almost all of the previously mentioned chemicals have been detected in groundwater near contaminant sites. High levels of exposure can cause headache, impaired cognition, and kidney toxicities

Dioxin: It has contaminated large areas of water and soil in the form of extremely toxic TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) through industrial accidents and through widespread use of the herbicide 2,4,5-T. Small amounts of TCDD were contained as a contaminant in herbicide manufacturing. The US Army used this herbicide, known as Agent Orange, extensively as a defoliant in Vietnam. TCDD is one of the most toxic synthetic substances known for laboratory animals: LD50 for male rats, 0.022 mg/kg; LD50 for female rats, 0.045 mg/kg; LD50 for female guinea pigs (the most sensitive species tested), 0.0006 mg/kg. In addition it is fetotoxic to pregnant rats at a dose of only 1/400 of the LD50, and has been shown to cause birth defects at levels of 1 to 3 ng/kg. TCDD is a proven carcinogen in both mice and rats, with the liver being the primary target. Although TCDD does not appear to be particularly acutely toxic to humans, chronic low-level exposure is suspected of contributing to reproductive abnormalities and carcinogenicity.

LECT. 6

OCCUPATIONAL TOXICANTS

Assessment of hazards in the workplace is a concern of occupational/industrial toxicology and has a history that dates back to ancient civilizations. The Greek historian Strabo, who lived in the first century AD, gave a graphic description of the arsenic mines in Pantus: "The air in mines is both deadly and hard to endure on account of the grievous odor of the ore, so that the workmen are doomed to a quick death." With the coming of the industrial revolution in the nineteenth century, industrial diseases increased, and new ones, such as chronic mercurialism caused by exposure to mercuric nitrate used in "felting" animal furs, were identified

Regulation of Exposure Levels

The goal of occupational toxicology is to ensure work practices that do not entail any unnecessary health risks. To do this, it is necessary to define suitable permissible levels of exposure to industrial chemicals, using the results of animal studies and epidemiological studies. These levels can be expressed by the following terms for allowable concentrations.

Threshold limit values (TLVs)

It refers to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effect. Because of wide variation in individual susceptibility, a small percentage of workers may experience discomfort from some substances at or below the threshold limit; a smaller percentage may be affected more seriously by aggravation of a preexisting condition or by development of an occupational illness. The basis on which the values are established may differ from substance to substance; protection against impairment of health may be a guiding factor for some, whereas reasonable freedom from irritation, narcosis, nuisance, or other forms of stress may form the basis for others. Three categories of TLVs follow:

1. Threshold limit value–time-weighted average (TLV-TWA)

It is the TWA concentration for a normal 8-hour workday or 40-hour workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

2. Threshold limit value–short-term exposure limit (TLV-STEL)

It is the maximal concentration to which workers can be exposed for a period up to 15 minutes continuously without suffering from (1) irritation, (2) chronic or irreversible tissue change, or (3) narcosis. of sufficient degree that would increase accident proneness, impair self-rescue, or materially work efficiency, provided that no more than four excursions per day are permitted, with at least 60 minutes between exposure periods, and provided that the daily TLV-TWA is not exceeded.

3. Threshold limit value–ceiling (TLV-C)

It is the concentration that should not be exceeded even instantaneously. For some substances—for instance, irritant gases—only one category, the TLV-ceiling, may be relevant. For other substances, two or three categories may be relevant. Biologic limit values (BLVs) represent limits of amounts of substances (or their affects) to which the worker may be exposed without hazard to health or well-being as determined by measuring the worker's tissues, fluids, or exhaled breath. The biologic measurements on which the BLVs are based can furnish two kinds of information useful in the control of worker exposure:

(1) measure of the worker's overall exposure.

(2) measure of the worker's individual and characteristic response.

Measurements of response furnish a superior estimate of the physiological status of the worker, and may consist of (1) changes in amount of some critical biochemical constituent, (2) changes in activity or a critical enzyme, and (3) changes in some physiological function. Measurement of exposure may be made by (1) determining in blood, urine, hair, nails, or body tissues and fluids the amount of substance to which the worker was exposed; (2) determining the amount of the metabolite(s) of the substance in tissues and fluids; and (3) determining the amount of the substance in the exhaled breath. The biologic limits may be used as an adjunct to the TLVs for air, or in place of them.

Immediately dangerous to life or health (IDLH) conditions pose a threat of severe exposure to contaminants, such as radioactive materials, that are likely to have adverse cumulative or delayed effects on health. Two factors are considered when establishingm IDLH concentrations. The worker must be able to escape (1) without loss of life or without suffering permanent health damage within 30 minutes and (2) without severe eye or respiratory irritation or other reactions that could inhibit escape. If the concentration is above the IDLH, only highly reliable breathing apparatus is allowed.

Routes of Exposure

The principal routes of industrial exposure are dermal and inhalation. Occasionally toxic agents may be ingested, if food or drinking water is contaminated. Exposure to the skin often leads to localized effects known as “occupation dermatosis” caused by either irritating chemicals or allergenic chemicals. Such effects include scaling، eczema, acne, pigmentation changes, ulcers, and neoplasia. Some chemicals may also pass through the skin; these include aromatic amines such as aniline and solvents such as carbon tetrachloride and benzene.

Toxic or potentially toxic agents may be inhaled into the respiratory tract where they may cause localized effects such as irritation (e.g., ammonia, chlorine gas), inflammation، necrosis, and cancer. Chemicals may also be absorbed by the lungs into the circulatory system, thereby leading to systemic toxicity (e.g., CO, lead).

LECT. 7

EXAMPLES OF INDUSTRIAL TOXICANTS

Learning Objectives

At the end of this chapter the student will be able to:

- Illustrate **examples of industrial toxicants**

Introduction

Carcinogen exposure is largely due to lifestyle, such as cigarette smoking, but occupation is an important source of exposure to carcinogens. [Table 1] lists some occupational chemical hazards and the cancers associated with them.

- **Cadmium** is a cumulative toxicant with a biologic half-life of up to 30 years in humans. More than 70% of the cadmium in the blood is bound to red blood cells; accumulation occurs mainly in the kidney and the liver, where cadmium is bound to metallothionein. In humans the critical target organ after long-term exposure to cadmium is the kidney, with the first detectable symptom of kidney toxicity being an increased excretion of specific proteins.
- **Chromium toxicity** results from compounds of hexavalent chromium that can be readily absorbed by the lung and gastrointestinal (GI) tract and to a lesser extent by the skin. Occupational exposure to chromium (Cr^{6+} causes dermatitis, ulcers on the hands and arms, perforation of the nasal septum (probably caused by chromic acid), inflammation of the larynx and liver, and bronchitis. Chromate is a carcinogen causing bronchogenic carcinoma; the risk to chromate plant workers for lung cancer is 20 times greater than that for the general population. Compounds of trivalent chromium are poorly absorbed. Chromium is not a cumulative chemical, and once absorbed, it is rapidly excreted into the urine.

Table 1. Some Occupational Hazards and Associated Cancers

Agent	Tumor Sites	Occupation
Asbestos	Lung, pleura, peritoneum	Miners, manufacturers, users
Arsenic	Skin, lung, liver	Miners and smelters, oil refinery, pesticide workers
Benzene	Hemopoietic tissue	Process workers, textile workers
Cadmium	Lung, kidney, prostate	Battery workers, smelters
Chloroethers	Lung	Chemical plant workers, process workers
Chromium	Lung, nasal cavity, sinuses	Process and production workers, pigment workers
Mustard gas	Bronchi, lung, larynx	Production workers
Naphthylamines	Bladder	Dyestuff makers and workers, Chemical workers, printers
Nickel	Lung, nasal sinuses	Smelters and process workers
Polycyclic aromatic hydrocarbons	Respiratory system, bladder	Furnace, foundry, shale, and gas workers; chimney sweeps
Radon, radium, uranium	Skin, lung, bone tissue, bone marrow	Medical and industrial chemists, miners
UV radiation	Skin	Outdoor exposure
X rays	Bone marrow, skin	Medical and industrial workers

- **Lead** is a ubiquitous toxicant in the environment, and consequently the normal body concentration of lead is dependent on environmental exposure conditions. Approximately %50of lead deposited in the lung is absorbed, whereas usually less than 10% of ingested lead passes into the circulation. Lead is not a major occupational problem today, but environmental pollution is still widespread. Lead interferes in the biosynthesis of porphyrins and heme, and several screening tests for lead poisoning make use of this interaction by monitoring either inhibition of the enzyme δ-aminolevulinic acid dehydratase (ALAD) or appearance in the urine of aminolevulinic acid (ALA) and coproporphorin (UCP). The metabolism of inorganic lead is closely related to that of calcium, and excess lead can be deposited in the bone where it remains for years. Inorganic lead poisoning can produce fatigue, sleep disturbances, anemia, colic, and neuritis. Severe exposure, mainly of children who have ingested lead, may cause encephalopathy, mental retardation, and occasionally, impaired vision. Organic lead has an affinity for brain tissue; mild poisoning may cause insomnia، restlessness, and GI symptoms, whereas severe poisoning results in delirium, hallucinations, convulsions, coma, and even death.
- **Mercury** is widely used in scientific and electrical apparatus, with the largest industrial use of mercury being in the chlorine-alkali industry for electrolytic production of chlorine and sodium hydroxide. Worldwide, this industry has been a major source of mercury contaminations. Most mercury poisoning, however, has been due to methylmercury, particularly

as a result of eating contaminated fish. Inorganic and organic mercury differ in their routes of entry and absorption. Inhalation is the principal route of uptake of metallic mercury in industry, with approximately 80% of the mercury inhaled as vapor being absorbed; metallic mercury is less readily absorbed by the GI route. The principal sites of deposition are the kidney and brain after exposure to inorganic mercury salts. Organic mercury compounds are readily absorbed by all routes. Industrial mercurialism produces features such as inflammation of the mouth, muscular tremors (hatters' shakes), psychic irritation, and a nephritic syndrome characterized by proteinuria. Overall, however, occupational mercurialism is not a significant problem today.

- **Benzene** was used extensively in the rubber industry as a solvent for rubber latex in the latter half of the nineteenth century. The volatility of benzene, which made it so attractive to the industry, also caused high atmospheric levels of the solvent. Benzene based rubber cements were used in the canning industry and in the shoe manufacturing industry. Benzene affects the hematopoietic tissue in the bone marrow and also appears to be an immunosuppressant. There is a gradual decrease in white blood cells, red blood cells, and platelets, and any combination of these signs may be seen. Continued exposure to benzene results in severe bone marrow damage and aplastic anemia. Benzene exposure has also been associated with leukemia.
- **Asbestos and other fibers** of naturally occurring silicates will separate into flexible fibers. Asbestos is the general name for this group of fibers. Chrysotile is the most important commercially and represents about 90% of the total used. Use of asbestos has been extensive, especially in roofing and insulation, asbestos cements, brake linings, electrical appliances, and coating materials. Asbestosis, a respiratory disease, is characterized by fibrosis, calcification, and lung cancer. In humans, not only is there a long latency period between exposure and development of tumors but other factors also influence the development of lung cancer. Cigarette smoking, for example, enhances tumor formation. Recent studies have shown that stomach and bowel cancers occur in excess in workers (e.g., insulation workers) exposed to asbestos. Other fibers have been shown to cause a similar disease spectrum, for instance, zeolite fibers.

LECT.8,9

TOXIC ACTION

Learning Objectives

At the end of this lecture the student will be able to:

1. Demonstrate Introduction about Water and soil toxicants
2. Mention the mechanisms of acute toxicity

INTRODUCTION

Acute toxicity of a chemical can be viewed from two perspectives. Acute toxicity may be the descriptor used as a qualitative indicator of an incident of poisoning. Consider the following statement: "methyl isocyanate gas, accidentally released from a chemical manufacturing facility in 1984, was acutely toxic to the residents of Bhopal, India". This statement implies that the residents of Bhopal were exposed to sufficiently high levels of methyl isocyanate over a relatively short time to result in immediate harm. High-level, short-term exposure resulting in immediate toxicity are all characteristics of acute toxicity. Alternatively, acute toxicity may represent a quantifiable characteristic of a material. For example, the statement: "the acute toxicity of methyl isocyanate, as measured by its LD50 in rats, is 140 mg/kg" defines the acute toxicity of the chemical.

❖ TOXICITY

❖ Acute Toxicity

Acute toxicity is defined as toxicity elicited as a result of short-term exposure to a toxicant. Incidences of acute toxicity in the environment are commonly associated with accident (i.e., derailment of a train resulting in leakage of a chemical into a river) or imprudent use of the chemical (i.e.,

aerial drift of a pesticide to nontarget areas). the acute toxicity of a chemical is commonly quantified as the LC50 or LD50[table 2].

Table 2. Ranking Scheme for Assessing the Acute Toxicity of Chemicals to Fish and Wildlife

Fish LC50 (mg/L)	Avian/Mammalian LD50 (mg/kg)	Toxicity Rank	Example Contaminant
>100	>5000	Relatively nontoxic	Barium
10–100	500–5000	Moderately toxic	Cadmium
1–10	50–500	Very toxic	1,4-Dichlorobenzene
<1	<50	Extremely toxic	Aldrin

❖ Mechanisms of Acute Toxicity

Environmental chemicals can elicit acute toxicity by many mechanisms. Provided below are example mechanisms that are particularly relevant to the types of chemicals that are more commonly responsible for acute toxicity in the environment at the present time.

Cholinesterase Inhibition. The inhibition of cholinesterase activity is characteristic of acute toxicity associated with organophosphate and carbamate pesticides. Cholinesterase inhibition in fish may occur following heavy rains in aquatic habitats adjacent to areas treated with the pesticides and subject to runoff from these areas. Acute toxicity to birds commonly occurs in birds that feed in areas following application of the pesticides.

Narcosis. A common means by which industrial chemicals elicit acute toxicity, particularly to aquatic organisms, is through narcosis. Narcosis occurs when a chemical accumulates in cellular membranes interfering with the normal function of the membranes. Typical responses to the narcosis are decreased activity, reduced reaction to external stimuli, and increased pigmentation (in fish). Chemicals that elicit toxicity via narcosis typically do not elicit toxicity at specific target sites and are sufficiently

lipophilic to accumulate in the lipid phase or the lipid-aqueous interface of membranes to sufficient levels to disrupt membrane function. Chemicals that induce narcosis include alcohols, ketones, benzenes, ethers, and aldehydes.

Physical Effects. Perhaps most graphic among recent incidents of environmental acute toxicity is the physical effects of petroleum following oil spills. Thousands of sea birds and mammals succumbed to the acute effects of the oil. Hypothermia is considered a major cause of death of oiled marine birds and mammals.

Toxicity to sea otters has been correlated to degree of oiling and is characterized by pulmonary emphysema(bubbles of air within the connective tissue of the lungs), gastric hemorrhages, and liver damage.

LECT. 10

ORGAN TOXICITY

Learning Objectives

At the end of this lecture the student will be able to:

3. Demonstrate Introduction about liver function.
4. Explain the mechanisms of hepatotoxicity.
5. Demonstrate the types of the liver injury.
6. Describe the Examples of hepatotoxicants.

INTRODUCTION

Liver Function

In the liver three main functions occur: storage, metabolism, and biosynthesis. Glucos is converted to glycogen and stored; when needed for energy, it is converted back to glucose. Fat, fat-soluble vitamins, and other nutrients are also stored in the liver. Fatty acids are metabolized and converted to lipids, which are then conjugated with proteins synthesized in the liver and released into the bloodstream as lipoproteins. The liver also nsynthesizes numerous functional proteins, such as enzymes and blood-coagulating factors. In addition the liver, which contains numerous xenobiotic metabolizing enzymes, is the main site of xenobiotic metabolism.

❖ TYPES OF LIVER INJURY

The types of injury to the liver depend on the type of toxic agent, the severity of intoxication, and the type of exposure, whether acute or chronic. The main types of liver damage are discussed briefly in this section. Whereas some types of damage—for example, cholestasis—are liver specific, others such as necrosis and carcinogenesis are more general phenomena.

1. Fatty Liver

Fatty liver refers to the abnormal accumulation of fat in hepatocytes. At the same time there is a decrease in plasma lipids and lipoproteins. Although many toxicants may cause lipid accumulation in the liver, the mechanisms may be different. Basically lipid accumulation is related to disturbances in either the synthesis or the secretion of lipoproteins. Excess lipid can result from an oversupply of free fatty acids from adipose tissues or, more commonly, from impaired release of triglycerides from the liver into the plasma. Triglycerides are secreted from the liver as lipoproteins (very low density lipoprotein, VLDL). As might be expected, there are a number of points at which this process can be disrupted. Some of the more important ones are as follows (Figure 10):

- Interference with synthesis of the protein moiety
- Impaired conjugation of triglyceride with lipoprotein
- Interference with transfer of VLDL across cell membranes
- Decreased synthesis of phospholipids
- Impaired oxidation of lipids by mitochondria
- Inadequate energy (adenosine triphosphate [ATP] for lipid and protein synthesis

The role that fatty liver plays in liver injury is not clearly understood, and fatty liver in itself does not necessarily mean liver dysfunction. The onset of lipid accumulation in the liver is accompanied by changes in blood biochemistry, and for this reason blood chemistry analysis can be a useful diagnostic tool.

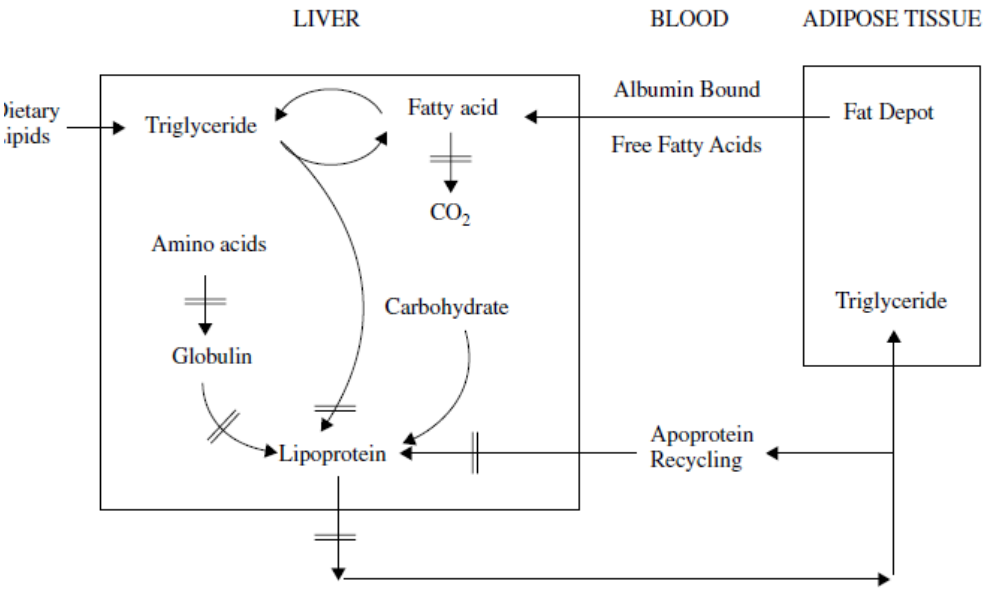


Figure 10. Triglyceride cycle in the pathogenesis of fatty liver. "=" are metabolic blocks.

2. Necrosis

Cell necrosis is a degenerative process leading to cell death. Necrosis, usually an acute injury, may be localized and affect only a few hepatocytes (focal necrosis), or it may involve an entire lobe (massive necrosis). Cell death occurs along with rupture of the plasma membrane, and is preceded by a number of morphologic changes such as cytoplasmic edema, dilation of the endoplasmic reticulum, disaggregation of polysomes, accumulation of triglycerides, swelling of mitochondria with disruption of cristae, and dissolution of organelles and nucleus. Biochemical events that may lead to these changes include binding of reactive metabolites to proteins and unsaturated lipids (inducing lipid peroxidation and subsequent membrane destruction) disturbance of cellular Ca+2 homeostasis, inference with metabolic pathways, shifts in Na+ and K+ balance, and inhibition of protein synthesis. Changes in blood chemistry resemble those seen with fatty liver, except they are quantitatively larger. Because of the regenerating capability of the liver, necrotic lesions are not

necessarily critical. Massive areas of necrosis, however, can lead to severe liver damage and failure.

3. Apoptosis

Apoptosis is a controlled form of cell death that serves as a regulation point for biologic processes and can be thought of as the counterpoint of cell division by mitosis. This selective mechanism is particularly active during development and senescence. Although apoptosis is a normal physiological process, it can also be induced by a number of exogenous factors, such as xenobiotic chemicals, oxidative stress, anoxia, and radiation. (A stimulus that induces a cell to undergo apoptosis is known as an apogen(If, however, apoptosis is suppressed in some cell types, it can lead to accumulation of these cells. For example, in some instances, clonal expansion of malignant cells and subsequent tumor growth results primarily from inhibition of apoptosis. Apoptosis can be distinguished from necrosis by morphologic criteria, using either light or electron microscopy. Toxicants, however, do not always act in a clear-cut fashion, and some toxicants can induce both apoptosis and necrosis either concurrently or sequentially.

4. Cholestasis

Cholestasis is the suppression or stoppage of bile flow, and may have either intrahepatic or extrahepatic causes. Inflammation or blockage of the bile ducts results in retention of bile salts as well as bilirubin accumulation, an event that leads to jaundice. Other mechanisms causing cholestasis include changes in membranes permeability of either hepatocytes or biliary canaliculi. Cholestasis is usually drug induced and is difficult to produce in experimental animals. Again, changes in blood chemistry can be a useful diagnostic tool.

5. Cirrhosis

Cirrhosis is a progressive disease that is characterized by the deposition of collagen throughout the liver. In most cases cirrhosis results from chronic chemical injury. The accumulation of fibrous material causes severe restriction in blood flow and in the liver's normal metabolic and detoxication processes. This situation can in turn cause further damage and eventually lead to liver failure. In humans, chronic use of ethanol is the single most important cause of cirrhosis, although there is some dispute as to whether the effect is due to ethanol alone or is also related to the nutritional deficiencies that usually accompany alcoholism.

6. Hepatitis

Hepatitis is an inflammation of the liver and is usually viral in origin; however, certain chemicals, usually drugs, can induce a hepatitis that closely resembles that produced by viral infections. This type of liver injury is not usually demonstrable in laboratory animals and is often manifest only in susceptible individuals. Fortunately, the incidence of this type of disease is very low.

7. Oxidative Stress

Oxidative stress has been defined as an imbalance between the prooxidant/antioxidant steady state in the cell, with the excess of prooxidants being available to interact with cellular macromolecules to cause damage to the cell, often resulting in cell death. Although the occurrence of reactive oxygen species in normal metabolism and the concept of oxidative stress was derived from these studies, it is apparent that oxidative stress can occur in almost any tissue, producing a variety of deleterious effects. To date, a number of liver diseases, including alcoholic liver disease, metal storage diseases، and cholestatic liver disease, have been shown to have an oxidative stress

component. Reactive oxygen and reactive nitrogen radicals can be formed in a number of ways (Figure 14.2), the former primarily as a by-product of mitochondrial electron transport. Superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl can all arise from this source. Other sources include monooxygenases and peroxisomes. If not detoxified، reactive oxygen species can interact with biological macromolecules such as DNA and protein or with lipids. Once lipid peroxidation of unsaturated fatty acids in phospholipids is initiated, it is propagated in such a way as to have a major damaging effect on cellular membranes. The formation, detoxication by superoxide dismutase and by glutathione-dependent mechanisms, and interaction at sites of toxic action are illustrated in [Figure 11].

8. Carcinogenesis

The most common type of primary liver tumor is hepatocellular carcinoma; other types include cholangiocarcinoma, angiosarcoma, glandular carcinoma, and undifferentiated liver cell carcinoma. Although a wide variety of chemicals are known to induce liver cancer in laboratory animals, the incidence of primary liver cancer in humans in the United States is very low. Some naturally occurring liver carcinogens are aflatoxin, cycasin, and safrole. A number of synthetic chemicals have been shown to cause liver cancer in animals، including the dialkylnitrosamines, dimethylbenzanthracene, aromatic amines such as2-naphthylamine and acetylaminofluorene, and vinyl chloride. In humans, the most noted case of occupation-related liver cancer is the development of angiosarcoma, a rare malignancy of blood vessels, among workers exposed to high levels of vinyl chloride in manufacturing plants.

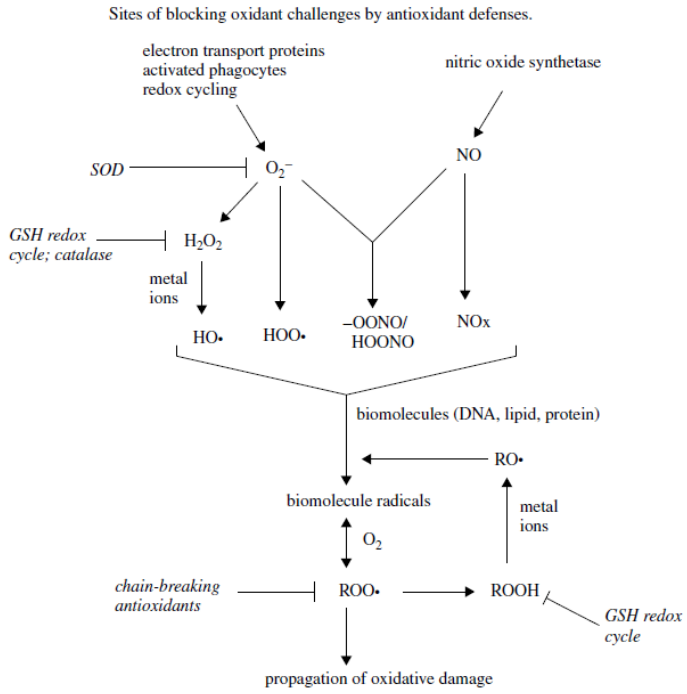


Figure 11. Molecular targets of oxidative injury.

❖ MECHANISMS OF HEPATOTOXICITY

Chemically induced cell injury can be thought of as involving a series of events occurring in the affected animal and often in the target organ itself:

1. The chemical agent is activated to form the initiating toxic agent.

2. The initiating toxic agent is either detoxified or causes molecular changes in the cell.

3. The cell recovers or there are irreversible changes.

4. Irreversible changes may culminate in cell death.

Cell injury can be initiated by a number of mechanisms, such as inhibition of enzymes, depletion of cofactors or metabolites, depletion of energy (ATP) stores, interaction with receptors, and alteration of cell membranes. In recent years attention has focused on the role of biotransformation of chemicals to

highly reactive metabolites that initiate cellular toxicity. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemical to highly reactive compounds, such as free radicals, carbenes, and nitrenes . These reactive metabolites can bind covalently to cellular macromolecules such as nucleic acids, proteins, cofactors, lipids, and polysaccharides, thereby changing their biologic properties. The liver is particularly vulnerable to toxicity produced by reactive metabolites because it is the major site of xenobiotic metabolism. Most activation reactions are catalyzed by the cytochrome P450 enzymes, and agents that induce these enzymes, such as phenobarbital and 3-methylcholanthrene, often increase toxicity. Conversely‹ inhibitors of cytochrome P450, such as SKF-525A and piperonyl butoxide, frequently decrease toxicity. Mechanisms such as conjugation of the reactive chemical with glutathione are protective mechanisms that exist within the cell for the rapid removal and inactivation of many potentially toxic compounds. Because of these interactions, cellular toxicity is a function of the balance between the rate of formation of reactive metabolites and the rate of their removal. Examples of these interactions are presented in the following discussions of specific hepatotoxicants.

- **EXAMPLES OF HEPATOTOXICANTS**
- Carbon Tetrachloride

Carbon tetrachloride has probably been studied more extensively, both biochemically and pathologically, than any other hepatotoxicant. It is a classic example of a chemical activated by cytochrome P450 to form a highly reactive free radical(Figure14.3). First, CCl4 is converted to the trichloromethyl radical (CCl3ž) and then to the trichloromethylperoxy radical (CCl3O2ž). Such radicals are highly reactive and generally have a small radius of action. For this reason the necrosis induced by CCl4 is most severe in the centrilobular liver cells that contain the highest concentration of the P450 isozyme responsible for

CCl4 activation. Typically free radicals may participate in a number of events (Figure 12), such as covalent binding to lipids, proteins, or nucleotides as well as lipid peroxidation. It is now thought that CCl3ž , which forms relatively stable adducts, is responsible for covalent binding to macromolecules, and the more reactive CCl3O2ž , which is formed when CCl3ž reacts with oxygen, is the prime initiator of lipid peroxidation. Lipid peroxidation (Figure 14.5) is the initiating reaction in a cascade of events‹ starting with the oxidation of unsaturated fatty acids to form lipid hydroperoxides, which then break down to yield a variety of end products, mainly aldehydes, which can go on to produce toxicity in distal tissues. For this reason cellular damage results not only from the breakdown of membranes such as those of the endoplasmic reticulum, mitochondria, and lysosomes but also from the production of reactive aldehydes that can travel to other tissues. It is now thought that many types of tissue injury, including inflammation, may involve lipid peroxidation.

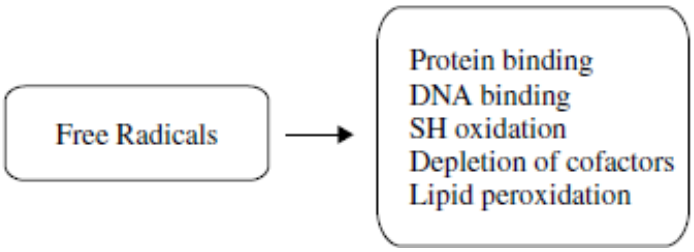


Figure 12. Summary of some toxic effects of free radicals.

❖ **Ethanol**

Alcohol-related liver diseases are complex, and ethanol has been shown to interact with a large number of molecular targets. Ethanol can interfere with hepatic lipid metabolism in a number of ways and is known to induce both inflammation and necrosis in the liver. Ethanol increases the formation of superoxide by Kupffer cells thus implicating oxidative stress in ethanol-induced liver disease. Similarly prooxidants (reactive oxygen species) are produced in

the hepatocytes by partial reactions in the action of CYP2E1, an ethanol-induced CYP isoform. The formation of protein adducts in the microtubules by acetaldehyde, the metabolic product formed from ethanol by alcohol dehydrogenase, plays a role in the impairment of VLDL secretion associated with ethanol.

LECT. 11

TOXICOLOGY OF THE NERVOUS SYSTEM

Learning Objectives

At the end of this article the student will be able to:

- Understanding about the effectiveness of different toxicants on the nervous system

NEUROTOXICITY

THE NERVOUS SYSTEM

Introduction

Most multicellular animals possess a nervous system. In every case the function of the nervous system is to receive information about the external and internal environment, integrate the information, and then coordinate a response appropriate to the environmental stimulus. In addition to these basic vital functions, the nervous system of higher organisms are responsible for feeling, thinking, and learning. All of the other organ systems of the body are subject to control by the nervous system; thus damage to this "master" system by toxicants can have far-reaching and even devastating effects[fig. 13,14].

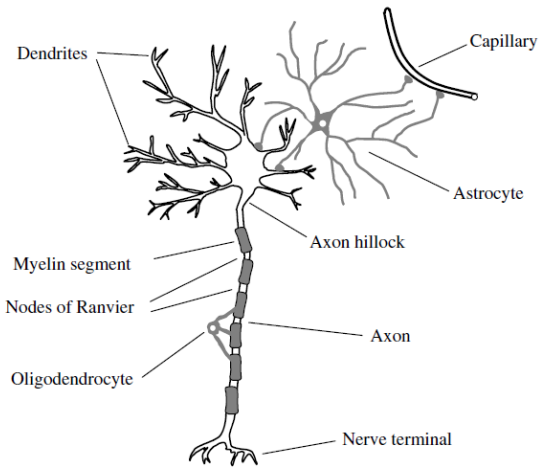


Figure 13. A neuron with accompanying astrocyte and myelinating oligodendrocyte.

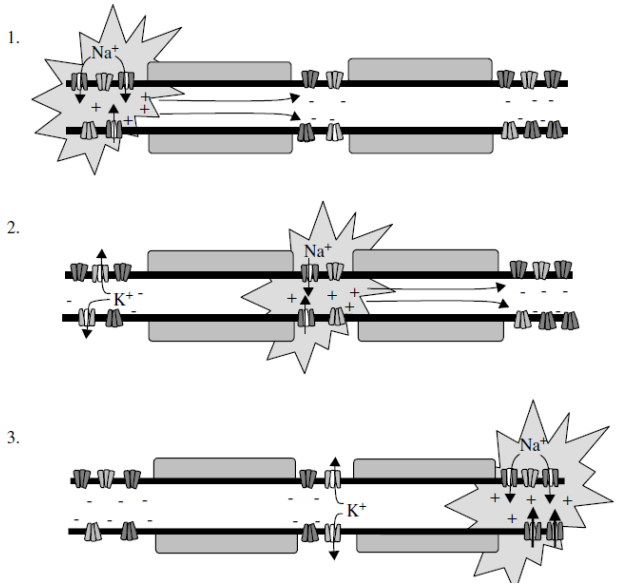


Figure 14. Saltatory conduction. Myelin acts as an insulator to prevent current loss as the action potential travels down the axon. Sodium and potassium channels are clustered at the Nodes of Ranvier, where there is no myelin. Action potentials jump from one node to the next, reducing the overall membrane area involved in conduction, and speeding up electrical transmission.

NEUROTOXICITY

It is a form of toxicity in which a biological, chemical, or physical agent produces an adverse effect on the structure or function of the central and/or peripheral nervous system. It occurs when exposure to a substance – specifically, a neurotoxin or neurotoxicant– alters the normal activity of the nervous system in such a way as to cause permanent or reversible damage to nervous tissue.[1] This can eventually disrupt or even kill neurons, which are cells that transmit and process signals in the brain and other parts of the nervous system. Neurotoxicity can result from organ transplants, radiation treatment, certain drug therapies, recreational drug use, exposure to heavy

metals, bites from certain species of venomous snakes, pesticides, certain industrial cleaning solvents,[4] fuels[5] and certain naturally occurring substances. Symptoms may appear immediately after exposure or be delayed. They may include limb weakness or numbness, loss of memory, vision, and/or intellect, uncontrollable obsessive and/or compulsive behaviors, delusions, headache, cognitive and behavioral problems and sexual dysfunction. Chronic mold exposure in homes can lead to neurotoxicity which may not appear for months to years of exposure. All symptoms listed above are consistent with mold mycotoxin accumulation.

In some cases the level or exposure-time may be critical, with some substances only becoming neurotoxic in certain doses or time periods. Some of the most common naturally occurring brain toxins that lead to neurotoxicity as a result of long term drug use are amyloid beta (Aβ), glutamate, dopamine, and oxygen radicals. When present in high concentrations, they can lead to neurotoxicity and death (apoptosis). Some of the symptoms that result from cell death include loss of motor control, cognitive deterioration and autonomic nervous system dysfunction. Additionally, neurotoxicity has been found to be a major cause of neurodegenerative diseases such as Alzheimer's disease (AD).

Neurotoxic agents

Amyloid beta

Amyloid beta (Aβ) was found to cause neurotoxicity and cell death in the brain when present in high concentrations. Aβ results from a mutation that occurs when protein chains are cut at the wrong locations, resulting in chains of different lengths that are unusable. Thus they are left in the brain until they are broken down, but if enough accumulate, they form plaques which are toxic to neurons. Aβ uses several routes in the central nervous system to cause cell death. An example is through the nicotinic acetylcholine receptor (nAchRs), which is a receptor commonly found along the surfaces of the cells that respond

to nicotine stimulation, turning them on or off. Aβ was found manipulating the level of nicotine in the brain along with the MAP kinase, another signaling receptor, to cause cell death. Another chemical in the brain that Aβ regulates is JNK; this chemical halts the extracellular signal-regulated kinases (ERK) pathway, which normally functions as memory control in the brain. As a result, this memory favoring pathway is stopped, and the brain loses essential memory function. The loss of memory is a symptom of neurodegenerative disease, including AD. Another way Aβ causes cell death is through the phosphorylation of AKT; this occurs as the phosphate group is bound to several sites on the protein. This phosphorylation allows AKT to interact with BAD, a protein known to cause cell death. Thus an increase in Aβ results in an increase of the AKT/BAD complex, in turn stopping the action of the anti-apoptotic protein Bcl-2, which normally functions to stop cell death, causing accelerated neuron breakdown and the progression of AD.

Glutamate

Glutamate is a chemical found in the brain that poses a toxic threat to neurons when found in high concentrations. This concentration equilibrium is extremely delicate and is usually found in millimolar amounts extracellularly. When disturbed, an accumulation of glutamate occurs as a result of a mutation in the glutamate transporters, which act like pumps to clear glutamate from the synapse. This causes glutamate concentration to be several times higher in the blood than in the brain; in turn, the body must act to maintain equilibrium between the two concentrations by pumping the glutamate out of the bloodstream and into the neurons of the brain. In the event of a mutation, the glutamate transporters are unable to pump the glutamate back into the cells; thus a higher concentration accumulates at the glutamate receptors. This opens the ion channels, allowing calcium to enter the cell causing excitotoxicity. Glutamate results in cell death by turning on the N-methyl-D-aspartic

acid receptors (NMDA); these receptors cause an increased release of calcium ions (Ca^{2+}) into the cells. As a result, the increased concentration of Ca^{2+} directly increases the stress on mitochondria, resulting in excessive oxidative phosphorylation and production of reactive oxygen species (ROS) via the activation of nitric oxide synthase, ultimately leading to cell death. Aβ was also found aiding this route to neurotoxicity by enhancing neuron vulnerability to glutamate.

Oxygen radicals

The formation of oxygen radicals in the brain is achieved through the nitric oxide synthase (NOS) pathway. This reaction occurs as a response to an increase in the Ca^{2+} concentration inside a brain cell. This interaction between the Ca^{2+} and NOS results in the formation of the cofactor tetrahydrobiopterin (BH4), which then moves from the plasma membrane into the cytoplasm. As a final step, NOS is dephosphorylated yielding nitric oxide (NO), which accumulates in the brain, increasing its oxidative stress. There are several ROS, including superoxide, hydrogen peroxide and hydroxyl, all of which lead to neurotoxicity. Naturally, the body utilizes a defensive mechanism to diminish the fatal effects of the reactive species by employing certain enzymes to break down the ROS into small, benign molecules of simple oxygen and water. However, this breakdown of the ROS is not completely efficient; some reactive residues are left in the brain to accumulate, contributing to neurotoxicity and cell death. The brain is more vulnerable to oxidative stress than other organs, due to its low oxidative capacity. Because neurons are characterized as postmitotic cells, meaning that they live with accumulated damage over the years, accumulation of ROS is fatal. Thus, increased levels of ROS age neurons, which leads to accelerated neurodegenerative processes and ultimately the advancement of AD.

Dopaminergic Neurotoxicity

Endogenous

The endogenously produced autotoxin metabolite of dopamine, 3,4-Dihydroxyphenylacetaldehyde (DOPAL), is a potent inducer of programmed cell death (apoptosis) in dopaminergic neurons. DOPAL may play an important role in the pathology of Parkinson's disease.

Drug induced

Certain drugs, most famously the pesticide and metabolite MPP+ (1-methyl-4-phenylpyridin-1-ium) can induce Parkinson's disease by destroying dopaminergic neurons in the substantia nigra. MPP+ interacts with the electron transport chain in the mitochondria to generate reactive oxygen species which cause generalized oxidative damage and ultimately cell death. MPP+ is produced by monoamine oxidase B as a metabolite of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), and its toxicity is particularly significant to dopaminergic neurons because of an active transporter on those cells that bring it into the cytoplasm. The neurotoxicity of MPP+ was first investigated after MPTP was produced as a contaminant in the pethidine synthesized by a chemistry graduate student, who injected the contaminated drug and developed overt Parkinson's within weeks. Discovery of the mechanism of toxicity was an important advance in the study of Parkinson's disease, and the compound is now used to induce the disease in research animals.

LECT. 12

NEPHROTOXICITY

Learning Objectives

At the end of this article the student will be able to:

- Mention the introduction about nephrotoxicity (function of the renal system).
- Describe the exambles of nephrotoxicity, cadmium and antimicrobial agents(cephalosporin).

RENAL SYSTEM

Introduction

Function of the Renal System

The primary function of the renal system is the elimination of waste products, derived either from endogenous metabolism or from the metabolism of xenobiotics. The latter function is discussed in detail in Chapter 10. The kidney also plays an important role in regulation of body homeostasis, regulating extracellular fluid volume, and electrolyte balance. Other functions of the kidney include the synthesis of hormones that affect metabolism. For example, 25-hydroxy-vitamin D3 is metabolized to the active form, 1,25- dihydroxy-vitamin D3. Renin, a hormone involved in the formation of angiotensin and aldosterone, is formed in the kidney as are several prostaglandins. While kidney toxicity could affect any of these functions, the effects used clinically to diagnose kidney damage are related to excretory function damage, such as increases in urinary glucose, amino acids, or protein, changes in urine volume, osmolarity, or pH. Similarly changes in blood urea nitrogen (BUN), plasma creatinine, and serum enzymes can be indicative of kidney damage. In animal studies of nephrotoxicity not only can histopathology be carried out but various biochemical parameters can be compared with those from untreated animals.

They include lipid peroxidation and covalent binding to tissue macromolecules[figure 15].

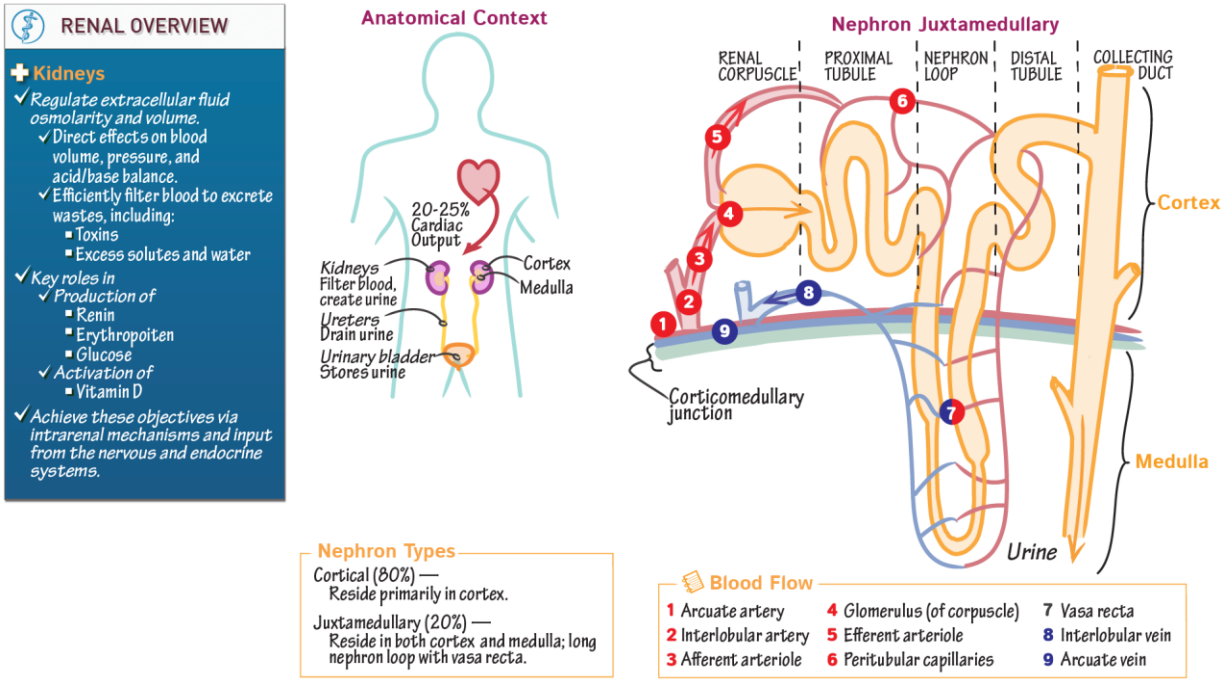


Figure 15. structure and function of the renal system

Nephrotoxicit

It is toxicity in the kidneys. It is a poisonous effect of some substances, both toxic chemicals and medications, on kidney function. There are various forms, and some drugs may affect kidney function in more than one way. Nephrotoxins are substances displaying nephrotoxicity.

EXAMPLES OF NEPHROTOXICANTS

- Cadmium

In humans, exposure to cadmium is primarily through food or industrial exposure to cadmium dust. In Japan, a disease called Itai-itai Byo is known to occur among women who eat rice grown in soils with a very high cadmium content. The disease is characterized by anemia, damage to proximal tubules, and severe bone and mineral loss. Cadmium is excreted in the urine mainly as a complex (CdMT) with the protein metallothionein

(MT). MT is a low molecular weight protein synthesized in the liver. It contains a large number of sulfhydryl groups that bind certain metals, including cadmium. The binding of cadmium by MT appears to protect some organs such as the testes from cadmium toxicity. At the same time, however, the complex may enhance kidney toxicity because the complex is taken up more readily by the kidney than is the free metal ion. Once inside the cell, it is thought that the cadmium is released, presumably by decomposition of the complex within the lysosomes. Cadmium has a long biological half-life, 10 to 12 years in humans; thus low-level chronic exposure will eventually result in accumulation to toxic concentrations.

❖ Antimicrobial agents(cephalosporin)

Several of the cephalosporins produce acute proximal tubular necrosis when given in large single doses. The degree of nephrotoxicity varies considerably among the individual cephalosporins toxicity is severe enough with some to restrict their use significantly.

❖ Renal Transport - Relationship to Toxicity of Cephaloridin

An understanding of the mechanisms of cephalosporin toxicity should begin with a brief review of the cellular mechanism of transport of the secreted organic anions .

Para-aminohippurate (PAR) is secreted across the proximal tubular cell after a primary active transport step at the antiluminal (blood) side. The resulting intracellular concentrations are considerably greater than those in the extracellular fluids, and favor subsequent movement of PAR down a concentration gradient into the tubular fluid, and thus into the urine. Pro benecid inhibits the secretion of PAR by a reduction of active transport at the antiluminal side, with a resulting lowering of both intracellular and tubular fluid concentrations Although there is certainly more than one organic anion transport system in the proximal tubule, the active transport of the various

penicillin and cephalosporin antibiotics appears to be related to that of PAR. Inhibition of secretion of the penicillins by PAR and that of the cephalosporins by PAR and the penicillins are suggestive of a common transport system for both the hippurates and the beta-Iactam antibiotics.

LECT. 13

ENDOCRINE TOXICOLOGY

Learning Objectives

At the end of this article the student will be able to:

- Mention the introduction about function of Endocrine system.
- Understandingthe Organizational versus Activational Effects of Endocrine Toxicants.

❖ ENDOCRINE SYSTEM

The endocrine system can be broadly described as an assemblage of organs (glands) that produce chemical messengers (hormones) that regulate various bodily functions. The bodily functions regulated by the endocrine system can be categorized as those involved in the maintenance of homeostasis and those involved in physiological progression.

Table 3. Processes Regulated by Some Hormones of the Endocrine System Susceptible to Disruption by Endocrine Toxicants

Hormone Group	Example	Origin	Regulated Process
Androgens	Testosterone	Testes, adrenals	Sexual differentiation, fertility, secondary sex characteristics, sexual function, libido
Estrogens	17β-Estradiol	Ovaries, testes	Sexual differentiation, fertility, secondary sex characteristics, bone density maintenance, blood coagulation
Glucocorticoids	Cortisol	Adrenals	Bone formation, wound healing, growth, development
Thyroid hormones	Thyroxine	Thyroid gland	Fetal brain and bone development, oxygen consumption, gut motility

Functions regulated by the endocrine system resulting in homeostasis include maintenance of the reproductive system, energy production, and metabolism. Functions regulated by the endocrine system resulting in physiological progression include fetal development, growth, and maturation. Endocrine processes related to physiological progression

historically have received the greatest attention in endocrine toxicology and will be emphasized in this chapter. Both the maintenance o homeostasis and the regulation of physiological progression require that the endocrine system detect signals, either external or internal, and transduce these signals to the appropriate target sites within the body. These target sites then respond in the appropriate manner to maintain homeostasis or institute change related to development, maturation, and so on. In many species these initial signals are of external origin. For example, many species initiate reproductive maturation in response to changes in environmental temperature and day length. Reproductively mature organisms often respond to external visual or olfactory stimuli produced by sexually receptive individual to initiate sexual behavior. The signal to be transduced by the endocrine system initiates in the central nervous system. In mammals, the hypothalamus commonly initiates the endocrine signaling pathway by secreting peptide hormones. These neuro-endocrine hormones can be rapidly synthesized, secreted, and degraded to allow near-instantaneous, short-lived responses to the stimulatory signal. Accordingly they can be present in the body in pulses and secretory rhythms that often contribute to their signaling function. For example, the hypothalamic peptide hormones “growth hormone releasing hormone(”GHRH) and somatostatin are secreted in an alternating pulsatile fashion. Both hormones target the pituitary gland, though GHRH stimulates and somatostatin inhibits growth hormone secretion by the pituitary. As a result the secretory pattern of the secondary hormone messenger in this cascade, growth hormone, is highly controlled. Disruption of this rhythm in rodent models can alter hepatic enzyme expression and other dynamic processes. Disruption of the growth hormone secretory rhythm associated with sleep has been shown to interfere with normal growth in children. Hormone secretory rhythms

have been associated with other physiological processes including sleep, sexual behavior, and ovulation. Endocrine signaling pathways from the central nervous system to the target organ typically occur along axes (Figure 16). An axis is defined by the endocrine glands that produce signaling hormones along the cascade (i.e., hypothalamic–pituitary–gonadal axis), and sometimes, a terminal target organ of the signaling pathway (i.e., hypothalamic– pituitary–gonadal–hepatic axis.(Endocrine signaling cascades offer several advantages over a single hormone signaling strategy. Cascades provide several sites at which the signal can be regulated thus ensuring maintenance of the appropriate endocrine signal (Figure 17).

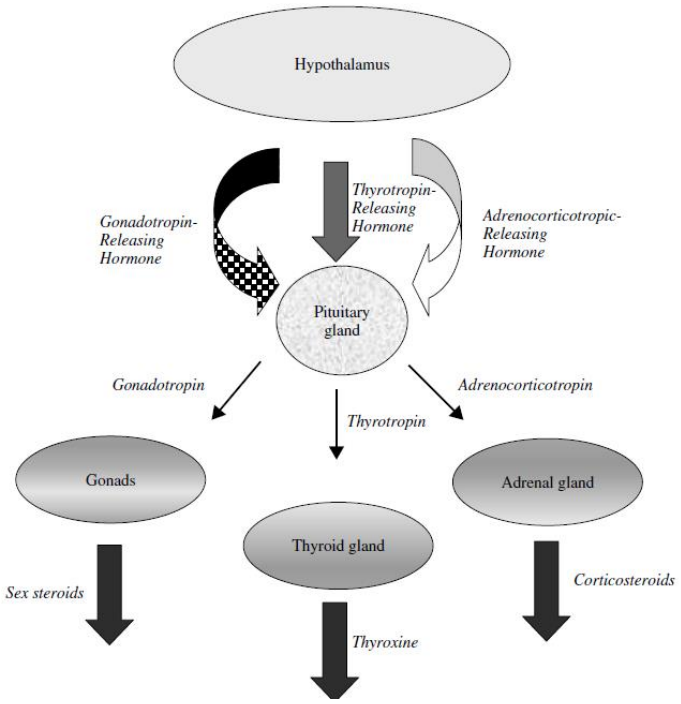


Figure 16. Some major neuro–endocrine axes that transduce endocrine signals to target organs. Neuro–endocrine signaling is initiated by the secretion of releasing hormones, or in some instances inhibiting hormones, that regulate secretion of the secondary hormone signal by the pituitary. Pituitary hormones then regulate secretion of the tertiary hormone, often a steroid hormone, by the appropriate endocrine gland. The tertiary hormones then stimulate gene transcription at target organs.

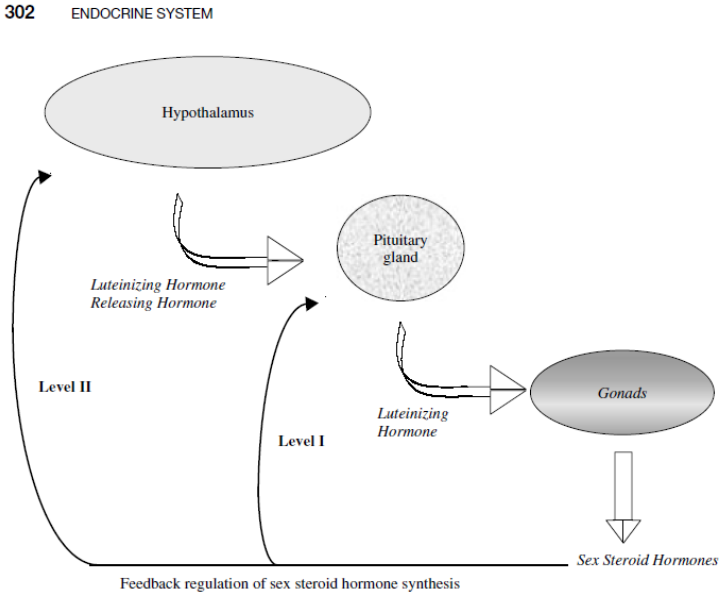


Figure 17. The hypothalamic–pituitary–gonadal axis. Endocrine signaling cascades provide multiple sites for regulation and ensure optimum signaling.

For example, testosterone is secreted by the testis but regulates its own secretion by acting upstream in the axis at the pituitary gland and hypothalamic gland. Signaling cascades also hormones are commonly the intermediate messengers along a signaling cascade, while the terminal hormone is often of nonpeptide origin (i.e., steroids). Peptide hormones offer advantages as intermediate messengers in that they can be rapidly synthesized and degraded (i.e., turned “on” and “off”). Peptide hormones also do not require cell entry to elicit activity but rather bind to cell surface receptors. This facilitates a rapid physiological response to the hormone. Steroid and other nonpeptide hormones are typically more stable, they are maintained in circulation at a relatively constant, physiologically appropriate level, they can be stored as precursor molecules or apolar conjugates, they can be mobilized as polar conjugates, and most often, they require cell entry to interact with its receptor and elicit a response. Accordingly the nonpeptide terminal

hormones offer the advantages of constant availability but lack the advantages of rapid modulation.

- **Organizational versus Activational Effects of Endocrine Toxicants**

Effects of receptor agonists or antagonists on endocrine related processes are often described as being either organizational or activational. An organizational effect of an endocrine toxicant is one that typically results from neonatal or prenatal exposure during which time hormones are directing various irreversible aspects of development. Accordingly the disrupting effect of the toxicant also is irreversible. These organizational

effects may be evident only later in life during maturation or reproduction.

Neonatal exposure to DES resulting in proliferation of epithelial cells of the reproductive tract at reproductive maturity is an example of an organizational effect of an endocrine toxicant. Organizational effects of endocrine toxicants have been of great concern to toxicologists and are the most difficult type of toxicity to diagnose owing to the temporal separation between exposure and effect. An activational effect of an endocrine toxicant occurs in the same time frame as the exposure and is the consequence of the toxicant disrupting the immediate role of a hormone in some physiological process. Activational effects are reversible following

cessation of exposure to the toxicant. For example, androgens contribute to maintenance of the prostate gland in the adult male. Exposure of adult males to an antiandrogen can result in a decrease in prostate size. Cessation of exposure to the antiandrogen then results in restoration of the prostate gland to its normal size.

❖ Inhibitors of Hormone Synthesis

Endocrine toxicants can elicit antihormone activity by lowering levels of endogenous hormone in the body. With steroid hormones, chemicals typically elicit this effect by inhibiting enzymes necessary for synthesis of the hormone. For example, the

cytochrome P450 enzyme CYP19 is responsible for the aromatization of testosterone to form 17β-estradiol. CYP19 inhibitors such as fadrozol, anastrozole, and letrozole, can lower endogenous 17β-estradiol levels resulting in de-feminization. Cytochrome P450s enzymes also are critical to various hydroxylation reactions that contribute to the synthesis of androgens and other steroid hormones and inhibition of these enzymes

can result in a variety of antisteroid hormone effects. For example, the agricultural and medicinal fungicides propiconazole, ketoconazole, and fenarimol are capable of inhibiting P450 enzymes and reducing synthesis and circulating levels of testosterone and other steroid hormones. Toxicological consequences of the lowering of endogenous steroid hormone levels are typically comparable to those effects elicited by antagonists of the hormone's receptor

❖ Inducers of Hormone Clearance

In most species, steroid and thyroid hormones are inactivated and cleared from the body by the same biotransformation processes that are involved in chemical detoxification. Predominant among the hormone biotransformation processes in vertebrates are hydroxylation, glucuronic acid conjugation, and sulfate conjugation. The thyroid hormones T3 and T4 are inactivated and cleared following sulfate and glucuronic acid conjugation, respectively. The glucuronosyl transferase enzymes that are

responsible for the elimination of T4 are induced following exposure to phenobarbitaltype inducers and Ah receptor ligands (see Chapter 9). Thus exposure to chemicals such as some dioxins and PCBs can result in enhanced clearance of thyroid hormone resulting in low circulating thyroid hormone levels. The resulting hypothyroid state can result in a variety of pathological conditions. In newborn infants, hypothyroidism is associated with cretinism. This organizational syndrome is characterized by mental retardation, short stature, and various neurological abnormalities. In children, hypothyroidism can cause delayed growth and mental development while advancing the onset of puberty in adolescents Hypothyroidism in adults results in various activational abnormalities including impaired cardiovascular, pulmonary, intestinal, and renal function. Chronic fatigue, lethargy, and difficulty in concentration are also associated with thypothyroidism in adults. Increased clearance of steroid hormones due to induction of hepatic biotransformation enzymes following chemical exposure often has been cited as a possible mechanism by which toxicants could lower circulating testosterone or 17β-estradiol levels.

While enhanced clearance of sex steroids has been demonstrated following chemical exposure and induction of hepatic biotransformation enzymes, elegant feedback control mechanisms tend to ensure that more hormone is produced and homeostasis is maintained (Figure 17.2). Enhanced clearance of sex steroids can contribute to endocrine disruption if the toxicity also results in impaired hormone synthesis (i.e,. gonadal toxicity or interference with the feedback control of hormone synthesis.(2,3,7,8-Tetrachlorodibenzodioxin appears to lower circulating sex steroid levels via this dual effect.

❖ Hormone Displacement from Binding Proteins

Steroid and thyroid hormones are typically distributed throughout the body while bound to serum-binding proteins such as sex hormone-binding globulin, corticosteroid-binding globulin, thyroxine-binding globulin (transthyretin), and albumin. Most steroid and thyroid hormones (>95%) are present in the blood reversibly bound to proteins. This bound hormone is not available for cell entry where it may interact with nuclear receptors or undergo inactivation/elimination reactions. Rather, the bound hormone serves as a reservoir from which hormone can be liberated (free hormone) for cell entry. Some xenobiotics can compete with hormones for binding to the blood proteins. As a result the circulating hormone reservoir can be depleted and free hormone becomes limited. A variety of phenolic compounds, including hydroxylated metabolites of polychlorinated biphenyls (PCBs), chlorophenols, chlorophenoxy acids, and nitrophenols, have been shown to interfere with thyroxine binding to thyroxine-binding globulin during in vitro experiments. In some instances compounds that displace thyroxine from the binding protein also have been shown to decrease circulating thyroxine levels in exposed animal models or in humans. In vitro experiments also have revealed that testosterone and 17β-estradiol can be displaced from sex hormone-binding globulin by some chemicals such as 4-nonylphenol, 4-tert-octylphenol, bisphenol A, O-hydroxybiphenyl, and pyrethroid insecticides. However, it is not clear whether these chemicals would significantly displace sex steroids from the binding globulin at concentrations typically measured in human blood.

LECT. 14

REPRODUCTIVE SYSTEM TOXICOLOGY

Learning Objectives

At the end of this article the student will be able to:

- Mention the introduction about function of Endocrine system.
- Describe incidents of endocrine toxicity(reproductive system).

INCIDENTS OF ENDOCRINE TOXICITY

Organizational Toxicity

In utero exposure to estrogens or antiandrogens has been shown, in animal models, to elicit a variety of organizational effects associated with development of the reproductive system. The best-described example of the organizational effects of a drug administered to humans involves the synthetic estrogen DES. DES was prescribed to over two million pregnant women in the United States between the 1940s and 1960s to prevent miscarriage. Offspring exposed to DES during fetal development experienced a variety of problems upon attainment of sexual maturity. DES daughters experience a significantly increased risk of clear cell adenocarcinoma of the vagina and cervix. DES daughters have increased risk of a variety of reproductive disorders including structural abnormalities of the reproductive tract, infertility, ectopic pregnancy, miscarriage, and pre-term delivery. Less is known of the risks faced by males exposed to DES during fetal development.

Hyperplasia and metaplasia of the prostatic ducts in DES sons also have been reported. The effects elicited by fetal exposure to DES appear to be largely the consequence of the estrogenic activity of this drug. Estrogens orchestrate organizational events during fetal development that

promote female reproductive tract development. Excess estrogen exposure resulting from DES treatment of either female or male fetuses resulted in permanent alterations, many of which became evident only upon attainment of reproductive maturity. Organizational effects on reproductive development resulting from perinatal exposure to endocrine toxicants of environmental origin also have been reported to occur. In 1973 a fire retardant containing polybrominated biphenyls (PBBs) was mistakenly added to cattle feed in Michigan. An estimated 4000 people subsequently were exposed to the PBBs by consuming dairy products derived from these cattle. PBBs are longlived chemicals that are stored in the fat of exposed individuals. PBBs have been reported to elicit endocrine toxicity-like symptoms in animal models consistent with hypothyroidism. For example, offspring from maternal rats provided PBBs during gestation and lactation showed signs of neurological deficit and growth retardation. Daughters of mothers that were exposed to PBBs during the Michigan incident were monitored for possible adverse effects on the female reproductive system. The initiation of menarche (menstruation) among these daughters correlated with the likely severity of PBB exposure. The most highly exposed daughters began menstruating approximately 1 year ahead of females that were less severely exposed. Early initiation of menarche is consistent with precocious puberty associated with hypothyroidism. The initiation of menarche also is under the regulation of 17β-estradiol and early initiation

of menarche may reflect an estrogen-type organizational effect of the PBBs during perinatal exposure.

❖ **Activational Toxicity**

Estrogenic Pharmaceuticals. Administration of estrogenic pharmaceuticals to children or adults can result in a variety of abnormalities associated largely with secondary sex characteristics that

are reversible upon cessation of drug treatment. Gynecomastia, the development of breast tissue in males, is often the consequence

of perturbations in the normal androgen/estrogen ratio. As discussed earlier in this chapter, prolonged administration of drugs with estrogenic or antiandrogenic activity can cause gynecomastia. Gynecomastia had been reported in the medical literature to occur as a result of frequent intercourse when an estrogen-containing cream was used as a vaginal lubricant and among morticans who applied estrogen-containing skin creams to corpses without the use of gloves. Similar to gynecomastia in adult males, activational toxicity from estrogenic drugs has been reported to cause pseudoprecocious puberty in children. Pseudoprecocious puberty is characterized by the development of some indicators of puberty (pubic o facial hair, morphological changes in sex organs, breast development, etc.) in preadolescent individuals. An outbreak of pseudoprecocious puberty was reported among a group of children ranging in age from 4 months to 2 years of age following application of a skin cream to treat dermatitis. Symptoms included pigmentation of the nipples, breast development, the presence of pubic hair, and vaginal discharge and bleeding among the females. Breast development also was reported in prepubertal boys following use of an estrogen-containing hair cream. These reports highlight the fact that dermal exposure can be adequate to attain a sufficient dose of endocrine-active compound to elicit adverse responses. In all of these cases the symptoms of endocrine toxicity resolved following cessation of exposure to the causative agent. Environmental Estrogens. Thelarche is defined as the development of breast tissue in preadolescent females (typically <8 years of age). Since 1979 physicians have monitored an epidemic level of thelarche on the island of Puerto Rico. The cause of thelarche in Puerto Rico is not known; however, evidence strongly implicates exposure to endocrine-disrupting

agents. Analyses of blood samples from thelarche and nonthelarche children for environmental chemicals with known estrogenic activity revealed that 68% of the thelarche children contained significantly high levels of several types of phthalate esters. Only a single nonthelarche child contained a significant amount of phthalate ester and only one type of phthalate ester was found in this individual. Phthalate esters are used as plasticizers and are ubiquitous environmental contaminants. They have been shown to cause a variety of endocrine-related effects in animal models and some phthalate esters have been shown to be estrogenic in vitro. The association between phthalate ester exposure and the high incidence of thelarche in Puerto Rico does not establish causality but has generated concern that environmental agents are

responsible for this condition. Kepone (chlordecone) is an organochlorine insecticide (Figure 17.5) that was manufactured in Hopewell, Virginia, from the mid-1960s to 1975. In 1975 the Center for Disease Control determined that employees of the manufacturing facility and other residents of Hopewell, totaling over 200 individuals, had been significantly contaminated with this insecticide. Exposed individuals reported a variety of symptoms. Foremost, among the symptoms of "Kepone sickness" were neurological disorders presenting as tremors, weight loss, and nervousness. However, subsequent evaluations revealed that males exposed to Kepone also experienced testicular dysfunction that was characteristic of estrogen exposure. Later laboratory studies demonstrated that Kepone was an estrogen receptor agonist, which could explain its adverse effects on the male reproductive system.

- **Hypothyroidism**

Hypothyroidism describes the clinical state arising from a deficiency in thyroid hormone. Toxicity resulting in hypothyroidism is manifested at several organ systems as Hypothyroidism can result from various causes

other than chemical toxicity including diseases of the hypothalamic–pituitary–thyroidal axis, iodine deficiency, and heritable defects in thyroid hormone production. Chemical agents that have historically been recognized for their ability to cause hypothyroidism include phenylbutazone, resorcinol, lithium, and para-aminosalicylic acid. Disruptions in thyroid hormone levels can occur through chemical-induced increases in the metabolic inactivation and elimination of the hormone. Chemicals that are capable of increasing the metabolic clearance of thyroid hormone include the polycyclic halogenated hydrocarbons (i.e., dioxins, furans, polychlorinated biphenyls, polybrominated biphenyls). A study reported in the New England Journal of Medicine suggested that environmental or occupational exposure to such chemicals can result in hypothyroidism in humans. The study consisted of a comparison of thyroid status in workers who were occupationally exposed to polybrominated biphenyls as compared to workers who were not exposed to any polyhalogenated hydrocarbons. Four of 35 exposed workers and none of 89 unexposed workers exhibited signs of hypothyroidism that included increased plasma levels of thyrotropin and decreased plasma levels of thyroxine. Thyrotropin is secreted by the pituitary gland and stimulates the thyroid gland to produce thyroxine (see Figure 17.1). The increase in thyrotropin and decrease in thyroxine is consistent with hypothyroidism caused by increased clearance of th thyroxine.

RESPIRATORY TRACT TOXICOLOGY

Learning Objectives

At the end of this article the student will be able to:

- Mention the introduction about respiratory system (function).
- Describe the exambles of nephrotoxicity, cadmium and antimicrobial agents(cephalosporin).

- **Function**

The nasal passages have an olfactory function, but with regard to inhaled toxicants they have primarily a defensive function and form the initial defensive barrier against inhaled toxicants. Since the nasal epithelium contains relatively high levels of xenobioticmetabolizing enzymes such as CYP and FMO isoforms, it can function as a detoxification site. However, as these enzymes, particularly CYP, may also activate toxicants, the nose is also a site for toxicant-induced lesions.

The trachea and bronchi likewise have a protective function. Mucous and serous cells secrete fluids that together comprise the mucus, which is moved toward the pharynx by the cilia of the ciliated cells. The movement of mucus serves to move entrapped particles toward the pharynx where they are eliminated by swallowing or expectoration. The mucus may also have other protective functions, protecting the epithelial cells by free radical scavenging and antioxidant properties. The Clara cells are known to contain high concentrations of xenobiotic metabolizing enzymes. The principal function of the lungs is gas exchange, providing O2 to the tissues and removing CO2. This gas exchange takes place in the alveoli. Because the lung has a large surface area and exchanges a significant volume of air (100,000–20,000 L/day for the average adult), the lung is the major interface between an organism the environment and any

toxicants present in the air. It is also significant, from an efficiency point of view, that the entire cardiac output goes to the lungs[fig. 18].

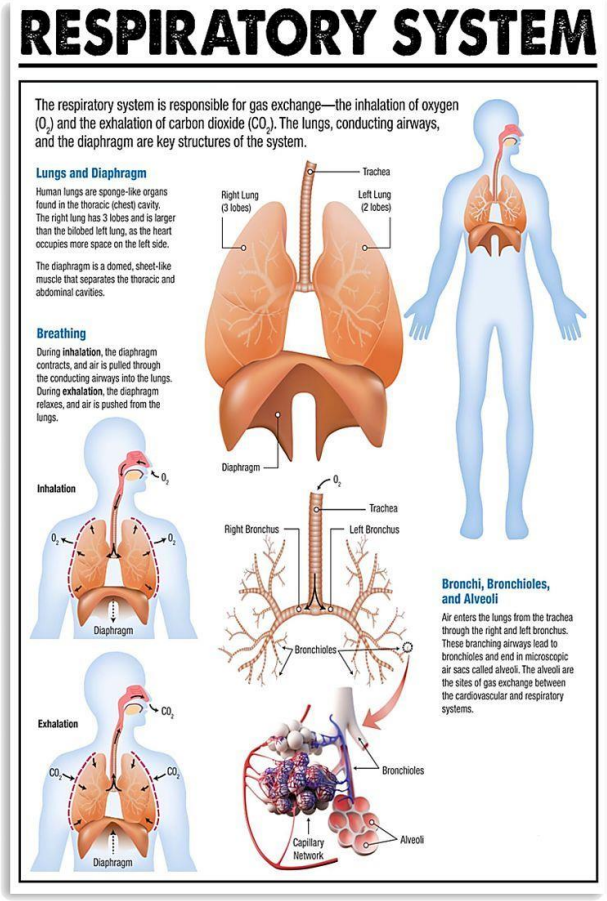


Figure 18. Structure and function of the respiratory system

SUSCEPTIBILITY OF THE RESPIRATORY SYSTEM

1. Nasal

The nasal epithelia are the first point of contact for respiratory toxicants. Because they contain xenobiotic metabolizing enzymes, they are susceptible to toxic effects caused by reactive intermediates.

2. Lung

In addition to being in direct contact with airborne toxicants, the entire body blood volume passes through the lung one to five times a minute, exposing the lung to toxicants and drugs within the systemic circulation. Thus the possibility of damage from both inhaled and circulating agents is

enormous. As with the liver and kidney, the lungs possess significant levels of many xenobiotic metabolizing enzymes and thus can play a large role in the activation and detoxication of exogenous chemicals.

TYPES OF TOXIC RESPONSE

Although many different agents may damage the lung, the patterns of cellular injury and repair are relatively constant, and most fall into one or more of the categories described below.

1.Irritation

Perhaps one of the most obvious and familiar chemical effects is irritation caused by volatile compounds such as ammonia or chlorine gas. Such irritation, especially if severe or persistent, results in constriction of the airways. Edema and secondary infection frequently follow severe or prolonged irritation. Such damage is known to result from exposure to agents such as ozone, nitrogen oxides, and phosgene.

2. Cell Necrosis

Severe damage to the cells lining the airways can result in increased cell permeability, followed by cell death.

3. Fibrosis

Fibrosis, or formation of collagenous tissue, was perhaps one of the earliest recognized forms of occupational diseases. Silicosis, resulting from inhalation of silica (SiO_2), is thought to involve first the uptake of the particles by macrophages and lysosomal incorporation, followed by rupture of the lysosomal membrane and release of lysosomal enzymes into the cytoplasm of the macrophages. Thus the macrophage is digested by its own enzymes. After lysis, the free silica is released to be ingested by fresh

macrophages, and the cycle continues. It is also thought that the damaged macrophages release chemicals that are instrumental in initiating the collagen formation in the lung. Fibrosis may become massive and impair

the respiratory function of the lung significantly. Asbestosis was recognized as long ago as 1907; however, the magnitude of the risk has become apparent only recently, primarily due to the increased incidence of lung cancer among asbestosis sufferers, especially those who are also cigarette smokers.

Both silicosis and asbestosis are thought to be premalignant conditions.

4. Emphysema

Emphysema is characterized by an enlargement of the air spaces with the destruction of the gas-exchange surface area. The loss of tissue and air-trapping capacity results in a distended lung that no longer effectively exchanges O2 and CO2. Although cigarette smoking is the major cause of emphysema, other toxicants can also cause this condition.

5. Allergic Responses

Numerous agents, including microorganisms, spores, dust, and chemicals, are known to elicit allergic responses resulting in constriction of the airways. Several diverse examples are farmer's lung from the spores of a mold that grows on damp hay, maple bark stripper's disease from spores of a fungus growing on maple trees, cheese washer's lung from penicillin spores, and mushroom picker's lung from the mushroom spores.

Byssinosis comes from the inhalation of cotton, flax, or hemp dusts. This condition, however, does not seem to result from bacterial or fungal exposure but from an apparent toxicant or allergen associated with the plant dusts.

6. Cancer

Perhaps the most severe response of the lung to injury is cancer, with the primary cause of lung cancer being cigarette smoking. Cigarette smoke contains many known carcinogens as well as lung irritants. Many of the polycyclic aromatic hydrocarbons, such as benzo(a)pyrene, can be

metabolized in the lung by pulmonary P450 enzymes to reactive metabolites capable of initiating cancer. In addition cigarette smoke contains numerous compounds that can act as tumor promoters. Asbestos is associated with two forms of cancer—lung cancer and malignant mesothelioma, a tumor of the cells covering the surface of the lung and the adjacent body wall.

7. Mediators of Toxic Responses

Most of the toxic responses summarized above involve a relatively small number of biochemical events related to oxidative injury, signaling pathways, and genotoxicity. Reactive oxygen species (ROS) such as superoxide anion (·O2) and hydroxyl radical (OH) are balanced by antioxidant enzymes and radical scavengers. Imbalance favoring ROS generation leads to lipid peroxidation, with resultant membrane damage, and DNA damage (genotoxicity) Changes in the concentration of growth factor and/or cytokine signaling molecules may lead to inflammation, proliferative responses, or apoptosis.

❖ EXAMPLES OF LUNG TOXICANTS REQUIRING ACTIVATION

Introduction

The activation of pulmonary toxicants falls into three main categories or mechanisms, depending either on the site of formation of the activated compound or on the nature of the reactive intermediate.

1. The parent compound may be activated in the liver, with the reactive metabolite then transported by the circulation to the lung. As would be expected, the activated compounds may lead to covalent binding and damage to both liver and lung tissue.

2. A toxicant entering the lung, either from inhaled air or the circulatory system, may be metabolized to the ultimate toxic compound directly within the lung itself. Although the total concentration of P450 is less in the lung than in the liver, the concentration varies considerably in the

different cell types, with the highest concentration being found in the nonciliated bronchiolar epithelial (Clara) cells of the terminal bronchioles. Because of this, the Clara cells are often a primary target for the effects of activated chemicals.

3. Another means of metabolic activation is the cyclic reduction/oxidation of the parent compound, resulting in high rates of consumption of NADPH and production of superoxide anion. Either the depletion of NADPH and/or the formation of reactive oxygen radicals could lead to cellular injury.

The following three chemicals serve to illustrate these three mechanisms of activation.

(Monocrotaline, Ipomeanol, Paraquat)

IMMUNE SYSTEM TOXICOLOGY

Learning Objectives

At the end of this article the student will be able to:

- Mention the introduction about the function of the immuney system .
- Describe the exambles of nephrotoxicity, cadmium and antimicrobial agents(cephalosporin).

- **Immune system**

Cells of the immune system include several types of leukocytes (white blood cells) (Table 19.1), which are derived from bone marrow. T lymphocytes, a subset of immune cells, undergo differentiation and maturation in the thymus. Leukocytes circulate throughout the body in blood and lymph and populate other lymphoid tissues including the spleen, lymph nodes (scattered throughout the body), tonsils, and adenoids, as well as aggregates of lymphoid tissue in the lung, gut, and skin, which are referred to as bronchus-, gut- and skin-associated lymphoid tissue (BALT, GALT, and SALT). Also immune cells can be recruited to almost any tissue in the body where there is injury or infection. Accumulation of leukocytes in tissues in response to injury is known as inflammation. Cytokines (e.g., interleukins, interferons, and chemokines), soluble mediators produced by immune cells as well as cells outside the immune system, control the maturation, differentiation, and mobilization of immune cells. Immune responses are divided into innate responses directed nonspecifically against foreign substances, and acquired responses directed against specific antigens.

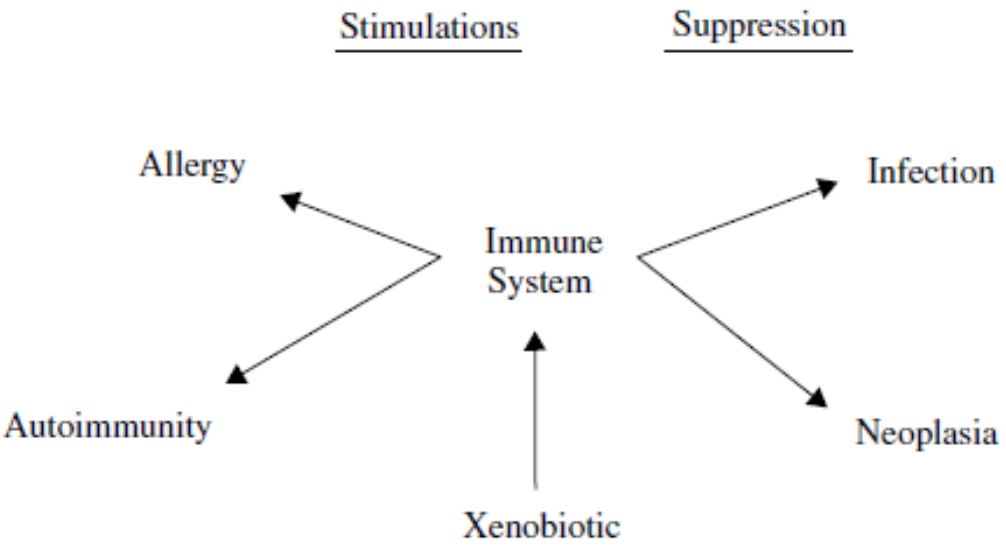


Figure 19. Potential consequences of immunotoxicity.

Table 4. Leukocytes

Granulocytes (polymorphonuclear leukocytes)
Neutrophils
Eosinophils
Basophils/mast cells[a]
Monocytes
Lymphocytes
Monocytes/macrophages[a]
Natural killer cells

[a]Found in blood/more activated form found in tissues.

There is considerable interaction between these two types of immunity. Innate immunity provides a rapid, although usually incomplete, antimicrobial defense. Granulocytes, natural killer cells, and macrophages are important mediators of innate immunity. Granulocytes have the capacity to phagocytize (engulf) infectious agents or other types of particles and to destroy or remove them from the tissue. They release a variety of soluble mediators that can kill invading organisms, increase vascular permeability, and recruit more leukocytes to the tissue. Natural killer cells are large granular lymphocytes that nonspecifically

kill tumor and virus-infected cells. Macrophages are also phagocytic, can release chemotactic and cytotoxic cytokines, and, when activated, can kill tumor or virus-infected cells. Mediators released fromall of these cells during the acute inflammatory response influence the development of acquired immune responses.

Acquired immunity specifically recognizes foreign substances (called antigens) and selectively eliminates them. On re-encountering the same antigen there is an enhanced response providing protection against reinfection. Vaccination against infectious agents is based on this principle. T lymphocytes and B lymphocytes (T cells and B cells) are the major players in acquired immunity (Figure 19.2). In both cases there are millions of different clones, groups of immune cells that have specific receptors for a particular antigen. When a cell encounters that specific antigen, clonal expansion occurs; that is, B and T cells with that particular specificity divide and differentiate and are thus activated to respond to the current crisis (e.g., infection). Memory cells develop that represent an enlarged clone of long-lived cells that are committed to respond rapidly, by clonal expansion, upon re-exposure to the same antigen.

B cells recognize native or denatured forms of proteins or carbohydrates in soluble, particulate, or cell-bound form. Activated B cells differentiate into plasma cells and produce antibodies, soluble proteins known as immunoglobulins (Ig), that circulate freely and react specifically with the invoking antigen. There are several classes (called isotypes) of Ig molecules—IgM, IgG, IgA, IgE, and IgD. IgM is the predominant antibody in the primary immune response (following initial exposure to an antigen). IgG usually appears later, following a primary infection, but is the predominant antibody in the response to subsequent exposures. IgE acts as a mediator of allergy and parasitic immunity.

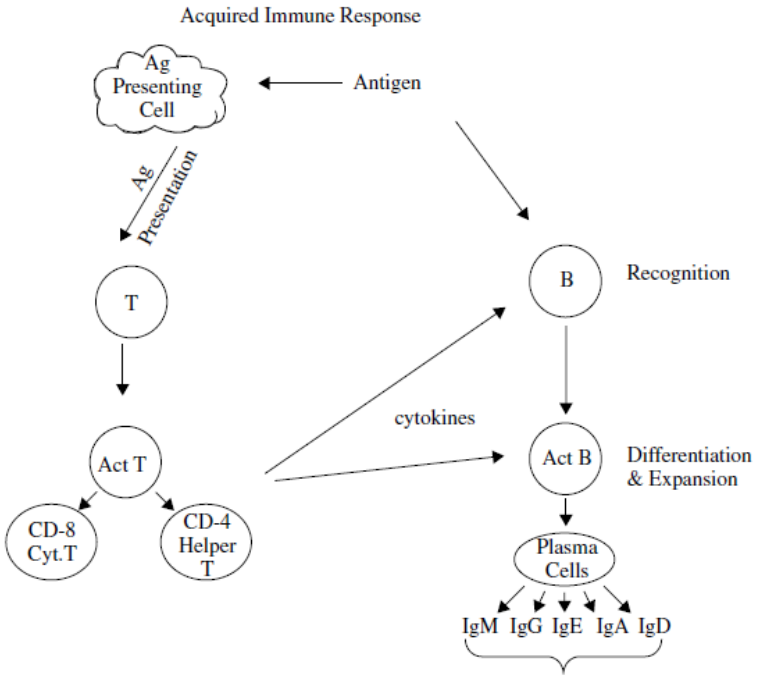


Figure 20. The acquired immune response. In response to a specific antigen there is clonal expansion of B cells and subsequent production of antibodies (Ig) specific for that antigen. Antigen presenting cells process and present antigen to T cells. Again there is clonal expansion of cells specific for that antigen.

IgA is found in secretions such as mucous, tears, saliva, and milk, as well as serum, and acts locally to block entrance of pathogens through mucous membranes. IgD is mainly membrane bound on B cells. Little is known about the

function of this isotype. It does not appear to have a unique role that affects host immunity. A given B cell will form antibody against just one single antigen; however, during the lifetime of this cell, it can switch to make a different class of antibody. Isotype switching is mediated by T helper cells. B cells recognize two types of antigen: Tindependent antigens, which activate the cell without T cell help (predominantly an IgM response), and T-dependent antigens, which required T cell help in order to activate B cells. Most antigens belong to this latter category. Antibodies that specifically recognize microbial antigens can, in combination with plasma proteins known as complement, lyse bacterial cells or

neutralize virus. Also microbes complexed with antibody are more readily phagocytized. T cells recognize antigen that is presented via an antigen-presenting cell (APC) such as macrophages or dendritic cells. APCs process and present short peptide fragments complexed with major histocompatibility (MHC) molecules on the surface of the APC. This processing and presentation is required for T cell activation. There are two major divisions of T cells that are distinguished by expression of different cell surface markers (CD4 and CD8).

CD-4 cells are also know as T-helper cells because they provide help for B cell activation. CD-8 cells are also known as cytotoxic T cells because they lyse cells expressing specific viral or tumor antigens. As indicated above the thymus plays a key role in T cell differentiation. Pre-T cells migrate from the bone marrow to the thymus. As relatively immature cells, T cells express both CD4 and CD8 molecules. As maturation progresses these cells undergo both positive and negative selection. During positive selection only cells that bind to MHC with a certain affinity survive. As a result of this process T cells become MHC restricted; that is, they will only respond to antigen presented in association with MHC. Cells that survive positive selection are potentially able to respond to self proteins. However, before T cells leave the thymus negative selection occurs during which self-reactive cells are removed or functionally inactivated. During the course of positive and negative selection CD4+ CD8+ cells down-regulate the expression of one of these molecules such that mature T cells express only CD4 or CD8. Mature T cells leave the thymus and populate secondary lymphoid organs.

❖ IMMUNE SUPPRESSION

Experimental studies in laboratory rodents have demonstrated that a diverse array of chemical exposures suppress immune function (Table 19.2). In addition a limited number of clinical and epidemiologic studies have reported suppression of immune function and/or increased frequency of infectious and/or neoplastic disease following exposure of humans to some of these agents. From

the description above it is clear there are a number of cellular and molecular targets for chemicals that act as immunosuppressants. Clearly, a chemical that disrupts cell proliferation would affect clonal expansion. Disruption of T cell maturation in the thymus is another potential mechanism for immune suppression. Chemicals may also interfere with receptor ligand binding at the cell surface and/or the cascade of signals that lead to transcription of genes responsible for generating and regulating the appropriate immune responses.

Table 5. Selected Examples of Immunosuppressive Agents

Drugs
Cyclosporin A, cyclophosphamide, glucocorticoids (Dexamethazone), azothioprine
Metals
Lead, cadmium, methylmercury, organotins[a]
Pesticides
Chlorodane[a], DDT[a], Dieldrin[a]
Industrial compounds
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated and polybrominated biphenyls (PCBs and PBBs), benzene, poly aromatic hydrocarbons[a]
Addictive substances
Cocaine, ethanol, opiates, cannabinoids, nicotine
Air pollutants
Environmental tobacco smoke, ozone, nitrogen dioxide
Microbial toxins
Aflatoxin,[b] ochratoxin A,[b] trichothecenes T-2 toxin[b]
Radiation
Ionizing, UV
Other
Asbestos, diethylstilbestrol (DES), dimethylnitrosamine

[a]Effects in humans are unknown; for all other compound without superscripts changes have been demonstrated in both rodents and humans.
[b]Effects in humans unknown, but veterinary clinicians have noted immunosuppression in livestock ingesting mycotoxins at levels below those that cause overt toxicity.

Because of the complexity of the immune system, tiered approaches to testing chemicals for immunosuppressive potential have been developed. Like other types of toxicity testing, the first level of the tier (Table 19.3) frequently relies solely on structural end points, including changes in the weight of thymus and other lymphoid organs, histopathology of these organs, or differential blood cell counts. This type of evaluation is convenient because it can be carried out along with an evaluation for other organ systems during routine toxicity testing using

one set of animals. However, although these nonfunctional endpoints may be effective in identifying gross (high dose) immunotoxic effects, they are not very accurate in predicting changes in immune function or alterations in susceptibility to challenge with infectious agents or tumor cells at lower chemical doses. Hence the first testing tier (Table 19.3) often includes functional end points designed to assess (1) antibody-mediated responses, (2(T-cell-mediated responses, and (3) NK cell activity. The most commonly used immune function assay in laboratory animals assesses the ability of a mouse or rat to respond to challenge with an antigen, usually sheep red blood cells (SRBC) (Figure 19.3). The response is assessed by determining the number of antigen specific antibody (IgM) forming cells (AFC) in the spleen (Jerne assay) or by assessing antigen specific antibodies in serum using an enzyme-linked immunosorbent assay (ELISA). Because the SRBC is a T-dependent antigen, T and B cells, as well as antigen presenting cells, must be functional to have a successful immunization. Suppression of this response is highly predictive of suppression of other immune function tests and also correlates well with tests that assess resistance to challenge with an infectious agent or tumor cells. The disadvantage to this test is that it usually requires a dedicated set of animals because of the antigen challenge. The most common approach has been to treat the animals for 14 to 28 days with the xenobiotic of interest, inject the antigen at the end of that exposure، and collect spleen or serum 4 to 5 days later. Unlike the tests for antibody-mediated immunity, tier 1 tests for cell-mediated immunity, and natural killer cell activity can be done ex vivo and do not require a dedicated set of animals. However, these tests focus on one cell type and are not as predictive of overall immunocompetence as the antibody assays. When immunosuppressive effects are noted in tier 1, an in-depth evaluation using more sophisticated tests may be carried out (tier 2, Table 19.4). This might include enumeration of lymphocyte subsets (B cells, total T cells, and CD4+ and CD8+) using flow cytometry or assessment of the IgM response to a T-independent antigen in an

effort to determine what portion of the immune response is the actual target. Unlike tier 1, tests of cell-mediated immunity in tier 2 require administration of an antigen and subsequent test for cytotoxic T cells (e.g., against an immunizing tumor cell) or a delayed type hypersensitivity response (similar to the response to a tuberculin test). In order to understand the mechanism's underlying immune suppression, a host of other tests can be carried out, including expression of an assortment of cytokines. Tier 2 also include host resistance models, tests in which an animal is exposed to a xenobiotic and then challenged with an infectious agent or tumor cells. This is considered the ultimate test for an adverse effect on the immune system. However, it should be noted that the amount of immune suppression that can be tolerated is greatly dependent on the dose and virulence of the challenging agent, as well as the genetics of the host. Manipulation of these variables can affect greatly results obtained in host resistance tests.