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TOXICOLOGY: A CURRENT CONCEPT & TRENDS

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ABSTRACT

Toxicology may be defined as "the study of the detection, occurrence, properties, effects, and regulation of toxic substances," although more expressive, do not resolve the difficulties. Toxicity itself cannot often, if ever, be defined as a single molecular event but is, rather, a cascade of events starting with exposure, proceeding through distribution and metabolism, and ending with interaction with cellular macromolecules (usually DNA or protein) and the expression of a toxic end point. Persistent pesticides applied directly to the soil have the possible to move from the soil into the water and thus enter the food chain from both soil and water. At the same time fertilizers leach out of the soil or runoff during rain events and flow into the natural water systems. Due to overlapping of mechanisms as well as use and chemical classes of toxicants, clear division into subjects of equal extent or significance is not possible. 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 exacting compound may vary with the gateway of access into the body, whether through the alimentary canal, the lungs, or the skin.

Key Words: Toxicology, Measurement, History, Carcinogens.

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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. By convention, toxicology also includes the study of harmful effects caused by physical phenomena, such as radiation of various kinds and noise. In practice, however, many complications exist beyond these simple definitions, both in bringing more precise meaning to what constitutes a poison and to the measurement of toxic effects. This sequence may be mitigated by excretion and repair. It is to the complications, and to the science behind them and their resolution, that this textbook is dedicated, particularly to the how and why certain substances cause disruptions in biologic systems that result in toxic effects. Taken together, these difficulties and their resolution circumscribe the perimeter of the science of toxicology. The study of toxicology serves society in many ways, not only to protect humans and the environment from the deleterious effects of toxicants but also to facilitate the development of more selective toxicants such as anticancer and other clinical drugs and pesticides [1-5].

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. Between these two limits there is a range of possible effects, from subtle long-term chronic toxicity to immediate lethality. Vinyl chloride may be taken as an example. It is a potent hepatic-toxicant 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. The importance of dose is well illustrated by metals that are essential in the diet but are toxic at higher doses. Thus iron, copper, magnesium, cobalt, manganese, and zinc can be present in the diet at too low a level (deficiency), at an appropriate level (maintenance), or at too high a level (toxic). The question of dose-response relationships is fundamental to toxicology [2-4].

The definition of a poison, or toxicant, also involves a qualitative biological aspect because a compound, toxic to one species or genetic strain, may be relatively harmless to another. For example, carbon tetrachloride, a potent hepatotoxicant in many species, is relatively harmless to the chicken [2-5].

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Certain strains of rabbit can eat *Belladonna* with impunity while others cannot. Compounds may be toxic under some circumstances but not others or, perhaps, toxic in combination with another compound but nontoxic alone. 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 outbreed and shows extensive genetic variation. Even the simplest measure of toxicity, the LD_{50} (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. LD_{50} values, as a result, vary markedly from one laboratory to another [3-6].

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 [2-7].

Relationship to Other Sciences, and History [5-8]

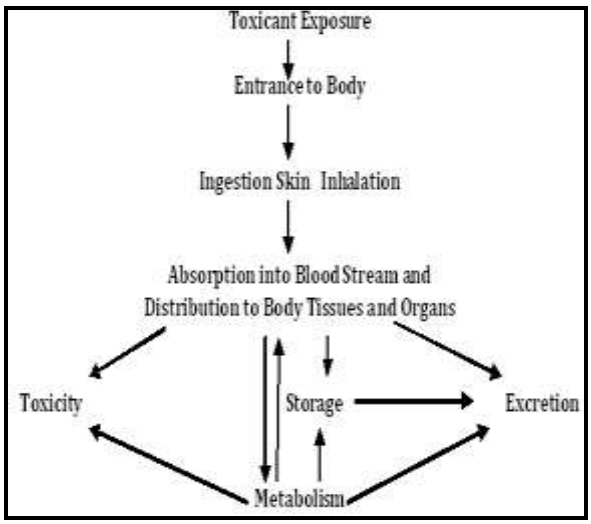

Figure 1: Fate and effect of toxicants in the body.

Modes of Toxic Action [6-9]:

This includes the consideration, at the fundamental level of organ, cell and molecular function, of all events leading to toxicity in vivo: uptake, distribution, metabolism, mode of action, and excretion. The term mechanism of toxic action is now more generally used to describe an important molecular event in the cascade of events leading from exposure to toxicity, such as the inhibition of acetyl cholinesterase in the toxicity of organophosphorus and carbamate insecticides.

Important aspects include the following:

- ***Biochemical and molecular toxicology*** consider events at the biochemical and molecular levels, including enzymes that metabolize xenobiotics, generation of reactive intermediates, interaction of xenobiotics or their metabolites with macromolecules, gene expression in metabolism and modes of action, and signalling pathways in toxic action.
- ***Behavioral toxicology*** deals with the effects of toxicants on animal and human behaviour, which is the final integrated expression of nervous function in the intact animal. This involves both the peripheral and central nervous systems, as well as effects mediated by other organ systems, such as the endocrine glands.
- ***Nutritional toxicology*** deals with the effects of diet on the expression of toxicity and with the mechanisms of these effects.
- ***Carcinogenesis*** includes the chemical, biochemical, and molecular events that lead to the large number of effects on cell growth collectively known as cancer.
- ***Teratogenesis*** includes the chemical, biochemical, and molecular events that lead to deleterious effects on development.
- ***Mutagenesis*** is concerned with toxic effects on the genetic material and the inheritance of these effects.
- ***Organ toxicity*** considers effects at the level of organ function (neurotoxicity, hepatotoxicity, nephrotoxicity, etc.).

Development of industrial Toxicology

At World War I, about 3000 to 4000 compounds were studied for acute toxicity for chemical warfare purposes, including phosgene ($COCl_2$) and bis (β-chloroethyl) sulfide). By the midt-1930s, large chemical companies established toxicological Laboratories, for example, DuPont, Dow and Union Carbide. In the US grants for industrial toxicology were given to US universities, for example, Harvard, University of Pittsburgh, New York University, University of Cincinnati and Johns Hopkins University. In 1949, the US FDA proposed procedures for 2-year studies in rats and 1-year studies in non-rodents as dogs. In 1959, the US FDA proposed a protocol for "lifetime" test with rats. The FDA also proposed a protocol for the three-generation reproductive study.

Measurement of Toxicants and Toxicity [7-11]:

These important aspects deal primarily with analytical chemistry, bioassay, and applied mathematics; they are designed to provide the methodology to answer certain critically important questions.

- Is the substance likely to be toxic?
- What is its chemical identifying?
- How much of it is present?
- How can we assay its toxic effect, and what is the minimum level at which this toxic effect can be detected?

A number of important fields are included:

- ***Analytical toxicology*** is a branch of analytical chemistry concerned with the identification and assay of toxic chemicals and their metabolites in biological and environmental materials.
- ***Toxicity testing*** involves the use of living systems to estimate toxic effects. It covers the gamut from short-term tests for genotoxicity such as the Ames test and cell culture techniques to the use of intact animals for a variety of tests from acute toxicity to lifetime chronic toxicity. Although the term "bioassay" is used properly only to describe the use of a living organism to quantitate the amount of a

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particular toxicant present, it is frequently used to describe any in vivo toxicity test.

- ***Toxicologic pathology*** is the branch of pathology that deals with the effects of toxic agents manifested as changes in subcellular, cellular, tissue, or organ morphology.
- *Structure-activity* studies are concerned with the relationship between the chemical and physical properties of a chemical and toxicity and, particularly, the use of such relationships as predictors of toxicity.
- ***Biomathematics and statistics*** relate to many areas of toxicology. They deal with data analysis, the determination of significance, and the formulation of risk estimates and predictive models.
- ***Epidemiology*** as it applies to toxicology is of great importance as it deals with the relationship between chemical exposure and human disease in actual populations rather than in experimental settings.

Applied Toxicology [11-14]:

This includes the various aspects of toxicology as they apply in the field or the development of new methodology or new selective toxicants for early application in the field setting.

- ***Clinical toxicology*** is the diagnosis and treatment of human poisoning.
- ***Veterinary toxicology*** is the diagnosis and treatment of poisoning in animals other than humans, particularly livestock and companion animals, but not excluding feral species. Other important concerns of veterinary toxicology are the possible transmission of toxins to the human population in meat, fish, milk, and other foodstuffs and the care and ethical treatment of experimental animals.
- ***Forensic toxicology*** concerns the medico legal aspects, including detection of poisons in clinical and other samples.
- ***Environmental toxicology*** is concerned with the movement of toxicants and their metabolites and degradation products in the environment and in food chains and with the effect of such contaminants on individuals and, especially, populations. Because of the large number of industrial chemicals and possibilities for exposure, as well as the mosaic of overlapping laws that govern such exposure, this area of applied toxicology is well developed.
- ***Industrial toxicology*** is a specific area of environmental toxicology that deals with the work environment and constitutes a significant part of *industrial hygiene*.

Chemical Use Classes:

This includes the toxicology aspects of the development of new chemicals for commercial use. In some of these use classes, toxicity, at least to some organisms, is a desirable trait; in others, it is an undesirable side effect. Use classes are not composed entirely of synthetic chemicals; many natural products are isolated and used for commercial and other purposes and must be subjected to the same toxicity testing as that required for synthetic chemicals. Examples of such natural products include the insecticide, pyrethrin, the clinical drug, digitalis, and the drug of abuse, cocaine.

- ***Agricultural chemicals*** include many compounds, such as insecticides, herbicides, fungicides, and rodenticides, in which toxicity to the target organism is a desired quality whereas toxicity to "non target species" is to be avoided. Development of such selectively toxic chemicals is one of the applied roles of comparative toxicology.
- ***Clinical drugs*** are properly the province of pharmaceutical chemistry and pharmacology. However, toxic side effects and testing for them clearly fall within the science of toxicology.
- ***Drugs of abuse*** are chemicals taken for psychological or other effects and may cause dependence and toxicity. Many of these are illegal, but some are of clinical significance when used correctly.
- ***Food additives*** are of concern to toxicologists only when they are toxic or being tested for possible toxicity.
- ***Industrial chemicals*** are so numerous that testing them for toxicity or controlling exposure to those known to be toxic is a large area of toxicological activity.
- ***Naturally occurring*** *substances* include many phytotoxins, mycotoxins, and minerals, all occurring in the environment. The recently expanded and now extensive use of herbal remedies and dietary supplements has become a cause of concern for toxicologists and regulators. Not only is their efficacy frequently dubious, but their potential toxicity is largely unknown.
- ***Combustion products*** are not properly a use class but are a large and important class of toxicants, generated primarily from fuels and other industrial chemicals.

Regulatory Toxicology these aspects, concerned with the formulation of laws, and regulations authorized by laws, are intended to minimize the effect of toxic chemicals on human health and the environment [12-16].

- ***Legal aspects*** are the formulation of laws and regulations and their enforcement. In the United States, enforcement falls under such government agencies as the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA). Similar government agencies exist in many other countries.
- ***Risk assessment*** is the definition of risks, potential risks, and the risk-benefit equations necessary for the regulation of toxic substances. Risk assessment is logically followed by *risk communication* and *risk management*.

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,

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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 have become increasingly important in recent years. Clearly, toxicology is preeminently an applied science, dedicated to the enhancement of the quality of life and the protection of the environment. It is also much more. Frequently the perturbation of normal life processes by toxic chemicals enables us to learn more about the life processes themselves.

Brief History of Toxicology

Much of the early history of toxicology [14-18] has been lost and in much that has survived toxicology is of almost incidental importance in manuscripts dealing primarily with medicine. Some, however, deal more specifically with toxic action or with the use of poisons for judicial execution, suicide or political assassination. Regardless of the paucity of the early record, and given the need for people to avoid toxic animals and plants, toxicology must rank as one of the oldest practical sciences.

The Egyptian papyrus, *Ebers*, dating from about 1500 BC, must rank as the earliest surviving pharmacopeia, and the surviving medical works of Hippocrates, Aristotle, and Theophrastus published during the period 400 to 250 BC all include some mention of poisons. It is clear, however, that since the 1960s toxicology has entered a phase of rapid development and has changed from a science that was largely descriptive to one in which the importance of mechanisms of toxic action is generally recognized. Since the 1970s, with increased emphasis on the use of the techniques of molecular biology, the pace of change has increased even further, and significant advances have been made in many areas, including chemical carcinogenesis and xenobiotic metabolism, among many others.

Dose-Response Relationships

As mentioned previously, toxicity is a relative event that depends not only on the toxic properties of the chemical and the dose administered but also on individual and interspecific variation in the metabolic processing of the chemical. The first recognition of the relationship between the dose of a compound and the response elicited has been attributed to Paracelsus. It is noteworthy that his statement includes not only that all substances can be toxic at some dose but that "the right dose differentiates a poison from a remedy," a concept that is the basis for pharmaceutical therapy. This concept is of significance because it implies that a *no observed effect level* (NOEL) can be determined and that this value can be used to determine the safe intake for food additives and contaminants such as pesticides. Although this is generally accepted for most types of chemicals and toxic effects, for chemical carcinogens acting by a genotoxic mechanism the shape of the curve is controversial and for regulatory purposes their effect is assumed to be a no-threshold phenomenon [16-20].

Sources of Toxic Compounds [19-25]

Exposure Classes, Toxicants in Air, Water, Soil, Domestic and Occupational Settings

Air Pollutants

Air pollution probably occurred as soon as humans started to use wood fires for heat and cooking. For centuries fire was used in such a way that living areas were filled with smoke. After the invention of the chimney, combustion products and cooking odors were removed from living quarters and vented outside. Later, when soft coal was discovered and used for fuel, coal smoke became a problem in the cities. By the thirteenth century, records show that coal smoke had become a nuisance in London, and in 1273 Edward I made the first antipollution law, one that prohibited the burning of coal while Parliament was in session: "Be it known to all within the sound of my voice, whosoever shall be found guilty of burning coal shall suffer the loss of his head." Despite this and various other royal edicts, however, smoke pollution continued in London. Increasing domestic and industrial combustion of coal caused air pollution to get steadily worse, particularly in large cities. During the twentieth century the most significant change was the rapid increase in the number of automobiles, from almost none at the turn of the century to millions within only a few decades. During this time few attempts were made to control air pollution in any of the industrialized countries until after World War II. In the United States the smog problem began to occur in large cities across the country, becoming especially severe in Los Angeles. In 1955 federal air pollution legislation was enacted, providing federal support for air pollution research, training, and technical assistance. Responsibility for the administration of the federal program now lies with the US Environmental Protection Agency (EPA). Technological interest since the mid-1950s has centered on automobile air pollution, pollution by oxides of sulfur and nitrogen, and the control of these emissions. Attention is also being directed toward the problems that may be caused by a possible greenhouse effect resulting from increased concentrations of carbon dioxide *(*CO_2*)* in the atmosphere, possible depletion of the stratospheric ozone layer, long-range transport of pollution, and acid deposition.

Types of Air Pollutants

What is clean air? Unpolluted air is a concept of what the air would be if humans and their works were not on earth, and if the air were not polluted by natural point sources such as volcanoes and forest fires. The true composition of "unpolluted" air is unknown because humans have been polluting the air for thousands of years. In addition there are many natural pollutants such as terpenes from plants, smoke from forest fires, and fumes and smoke from volcanoes.

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 is carbon monoxide (CO), hydrocarbons, hydrogen sulfide *(*H_2S*)*

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Particulate *Pollutants*

Fine solids or liquid droplets can be suspended in air. Some of the different types of particulates are defined as follows:

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

Sources of Air Pollutants [24-28]

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. Dust storms are a common source of particulate matter in many parts of the world, and oceans produce aerosols in the form of salt particles. Plants and trees are a major source of hydrocarbons on the planet, and the blue haze that is so familiar over forested mountain areas is mainly from atmospheric reactions with volatile organics produced by the trees. Plants also produce pollen and spores, which cause respiratory problems and allergic reactions.

Anthropogenic Pollutants

These substances come primarily from three sources:

- combustion sources that burn fossil fuel for heating and power, or exhaust emissions from transportation vehicles that use gasoline or diesel fuels;
- industrial processes; and
- Mining and drilling.

The principal pollutants from combustion are fly ash, smoke, sulfur, and nitrogen oxides, as well as CO and CO_2. 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 CO_2 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. Since the mid-1960s there has been significant progress in reducing exhaust emissions, particularly with the use of low-lead or no-lead gasoline as well as the use of oxygenated fuels-for example, fuels containing ethanol or MTBE (methyl *t*-butyl ether).

Industries may emit various pollutants relating to their manufacturing processes [27-30]:

Acids (sulfuric, acetic, nitric, and phosphoric), solvents and resins, gases (chlorine and ammonia), and metals (copper, lead, and zinc).

Indoor Pollutants

In general, the term "indoor air pollution" refers to home and non factory 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.

Although indoor air pollution has increased in developed nations because of tighter building construction and the use of building materials that may give off gaseous chemicals, 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

Most of the information on the effects of air pollution on humans comes from acute pollution episodes such as the ones in Donora and London. Illnesses may result from chemical irritation of the respiratory tract, with certain sensitive subpopulations being more affected:

- very young children, whose respiratory and circulatory systems are poorly developed,
- the elderly, whose cardio respiratory systems function poorly, and
- People with cardio respiratory diseases such as asthma, emphysema, and heart disease. Heavy smokers are also affected more adversely by air pollutants. In most cases the health problems are attributed to the combined action of particulates and sulphur dioxides *(SO_2)*; no one pollutant appears to be responsible.

Carbon Monoxide

Carbon monoxide combines readily with hemoglobin (Hb) to form carboxy hemoglobin (COHb), thus preventing the transfer of oxygen to tissues. The affinity of hemoglobin for CO is approximately 210 times its affinity for oxygen. A blood concentration of 5% COHb, equivalent to equilibration at approximately 45 ppm CO, is associated with cardiovascular effects. Concentrations of 100 ppm can cause headaches, dizziness, nausea, and breathing difficulties. An acute concentration of 1000 ppm is invariably fatal. Carbon monoxide levels during acute traffic congestion have been known to be as high as 400 ppm; in addition, people who smoke elevate their total body burden of CO as compared with nonsmokers. The effects of low concentrations of CO over a long period are not known, but it is possible that heart and respiratory disorders are exacerbated.

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

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inner respiratory tract, where they are not effectively removed. In the respiratory tract, SO_2 combines readily with water to form sulfurous acid, resulting in irritation of mucous.

Principal Air Pollutants, Sources, and Effects [30-34]

Main component of acid deposition Damage to vegetation, materials Irritating to lungs, chronic bronchitis Nitrogen oxides Automobile emissions Pulmonary edema, impairs lung defenses Fossil fuel power plants Important component of photochemical smog and acid deposition Carbon monoxide Motor vehicle emissions Burning fossil fuels Incomplete combustion Combines with hemoglobin to form carboxy hemoglobin, poisonous Asphyxia and death Carbon dioxide Product of complete combustion May cause "greenhouse effect" Ozone *(O_3)* Automobile emissions Damage to vegetation Photochemical smog Lung irritant Hydrocarbons, C*x*H*y* Smoke, gasoline fumes Contributes to photochemical smog Cigarette smoke, industry.

Nitrogen Oxides

Nitrogen dioxide *(NO_2)*, a gas found in photochemical smog, is also a pulmonary irritant and is known to lead to pulmonary edema and hemorrhage. The main issue of concern is its contribution to the formation of photochemical smog and ozone, although nitrogen oxides also contribute to acid deposition.

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.

NO2 + UV light −−− NO + Ož

Ož + O_2 −−− O_3

At this point it is worth distinguishing between "good" and "bad" ozone. *Tropospheric ozone* occurs from 0 to 10 miles above the earth's surface, and is harmful. *Stratospheric ozone*, located about 30 miles above the earth's surface, is responsible for filtering out incoming UV radiation and thus is beneficial. It is the decrease in the stratospheric ozone layer that has been of much concern recently. It is estimated that a 1% decrease in stratospheric ozone will increase the amount of UV radiation reaching the earth's surface by 2% and cause a 10% increase in skin cancer. Major contributors to damage to stratospheric ozone are thought to be the chlorofluorocarbons (CFCs). Chlorine is removed from the CFC compounds in the upper atmosphere by reaction with UV light and is then able to destroy the stratospheric ozone through self-perpetuating free radical reactions.

Cl + O_3 −−−ClO + O_2

ClO + O −−−Cl + O_2

Before being inactivated by nitrogen dioxide or methane, each chlorine atom can destroy up to 10,000 molecules of ozone. Use of CFC compounds is now being phased out by international agreements.

Hydrocarbons (HCs) or Volatile Organic Compounds (VOCs)

These are derived primarily from two sources: approximately 50% are derived from trees as a result of the respiration process (biogenic); the other 45% to 50% comes from the combustion of fuel and from vapor from gasoline. Many gasoline pumps now have VOC recovery devices to reduce pollution.

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. It is estimated that approximately 20% of the total body burden of lead comes from inhalation.

Solid Particles

Dust and fibers from coal, clay, glass, asbestos, and minerals can lead to scarring or fibrosis of the lung lining. Pneumoconiosis, a condition common among coal miners that breathe coal dust, silicosis caused by breathing silica-containing dusts, and asbestosis from asbestos fibers are all well-known industrial pollution diseases.

Environmental Effects [34-37]:

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. Air pollution can also result in measurable effects on forest ecosystems, such as reduction in forest growth, change in forest species, and increased susceptibility to forest pests. High-dose exposure to pollutants, which is associated with point source emissions such as smelters, frequently results in complete destruction of trees and shrubs in the surrounding area.

Domestic Animals

Although domestic animals can be affected directly by air pollutants, the main concern is chronic poisoning as a result of ingestion of forage that has been contaminated by airborne pollutants. Pollutants important in this connection are

Examples of Air Pollution Injury to Vegetation

Pollutant Symptoms

Sulfur dioxide Bleached spots, interveinal bleaching

Ozone Flecking, stippling, bleached spotting

Peroxy acetyl nitrate (PAN) Glazing, silvering, or bronzing on lower leaf surfaces

Nitrogen dioxide White or brown collapsed lesion near leaf margins

Hydrogen fluoride Tip and margin burns, dwarfing arsenic, lead, and molybdenum. Fluoride emissions from industries producing phosphate fertilizers and derivatives have damaged cattle throughout the world. The raw material, phosphate rock, can contain up to 4% fluoride, some of which is released into the air and water. Farm animals, particularly cattle, sheep, and swine, are susceptible to fluoride toxicity (fluorosis), which is characterized by

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mottled and soft teeth, and ostero-fluoritic bone lesions, which lead to lameness and, eventually, death.

Materials and Structures

Building materials have become soiled and blackened by smoke, and damage by chemical attack from acid gases in the air has led to the deterioration of many marble statues in Western Europe. Metals are also affected by air pollution; for example, SO_2 causes many metals to corrode at a faster rate. Ozone is known to oxidize rubber products, and one of the effects of Los Angeles smog is cracking of rubber tires. Fabrics, leather, and paper are also affected by SO_2 and sulfuric acid, causing them to crack, become brittle, and tears more easily.

Atmospheric Effects

The presence of fine particles (0.1–1.0 mm in diameter) or NO_2 in the atmosphere can result in atmospheric haze or reduced visibility due to light scattering by the particles. The major effect of atmospheric haze has been degradation in visual air quality and is of particular concern in areas of scenic beauty, including most of the major national parks such as Great Smoky Mountain, Grand Canyon, Yosemite, and Zion Parks. There is also concern over the increase in CO_2 in the atmosphere because CO_2 absorbs heat energy strongly and retards the cooling of the earth. This is often referred to as the greenhouse effect; theoretically an increase in CO_2 levels would result in a global increase in air temperatures. In addition to CO_2, other gases contributing to the greenhouse effect include methane, CFCs, nitrous oxide, and ozone.

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. In the eastern United States, the acids in acid rain are approximately 65% sulfuric, 30% nitric, and 5% other, whereas in the western states, 80% of the acidity is due to nitric acid. Many lakes in northeastern North America and Scandinavia have become so acidic that fish are no longer able to live in them. The low pH not only directly affects fish but also contributes to the release of potentially toxic metals, such as aluminum, from the soil. The maximum effect occurs when there is little buffering of the acid by soils or rock components.

Maximum fish kills occur in early spring due to the "acid shock" from the melting of winter snows. 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. Much of the area in eastern Canada and the northeastern United States is covered by thin soils with low acid neutralizing capacity. In such areas the lakes are more susceptible to the effects of acid deposition leading to a low pH and high levels of aluminum, a combination toxic to many species of fish.

A second area of concern is that of reduced tree growth in forests. The leaching of nutrients from the soil by acid deposition may cause a reduction in future growth rates or changes in the type of trees to those able to survive in the altered environment. In addition to the change in soil composition, there are the direct effects on the trees from sulfur and nitrogen oxides as well as ozone.

Water and Soil Pollutants

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, Domestic and municipal wastes, both from sewage and from disposal of chemicals, are another major source of chemical pollutants. At the turn of the twentieth century, municipal wastes received no treatment and were discharged directly into rivers or oceans. Even today, many older treatment plants do not provide sufficient treatment, especially plants in which both storm water and sewage are combined. In addition to organic matter, pesticides, fertilizers, detergents, and metals are significant pollutants discharged from urban areas. Contamination of soil and water also results from the use of pesticides and fertilizers. Pollution from petroleum compounds has been a major concern since the mid-1960s. In 1967 the first major accident involving an oil tanker occurred.

The *Torrey Canyon* ran onto rocks in the English Channel, spilling oil that washed onto the shores of England and France. It is estimated that at least 10,000 serious oil spills occur in the United States each year. In addition, flushing of oil tankers plays a major role in marine pollution. Other sources, such as improper disposal of used oil by private car owners and small garages, further contribute to oil pollution.

Examples of Pollutants [35-40]

Metals that are of environmental concern fall into three classes:

- Metals that are suspected carcinogens,
- Metals that move readily in soil, and

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- 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 the 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. Lead and associated toxic effects

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. 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.

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. By 1970, at least 107 deaths had been attributed to mercury poisoning.

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.

Chemical Carcinogenesis [34-41]

General Aspects of Cancer

Carcinogenesis is the process through which cancer develops. Chemical carcinogenesis is the study of the mechanisms through which chemical carcinogens induce cancer and also involves the development /utilization of experimental systems aimed at determining whether a substance is a potential human carcinogen. An important aspect of toxicology is the identification of potential human carcinogens. To begin to appreciate the complexity of this subject, it is important to first have some understanding of cancer and its etiologies.

Cancer is not a single disease but a large group of diseases, all of which can be characterized by the uncontrolled growth of an abnormal cell to produce a population of cells that have acquired the ability to multiply and invade surrounding and distant tissues. It is this invasive characteristic that imparts its lethality on the host. Epidemiology studies have revealed that the incidence of most cancers increase exponentially with age Epidemiologist have interpreted this exponential increase in cancer incidence to denote that three to seven critical mutations or “hits” within a single cell are required for cancer development. Molecular analyses of human tumors have confirmed the accumulation of mutations in critical genes in the development of cancer. These mutations can be the result of imperfect DNA replication/repair, oxidative DNA damage, and/or DNA damage caused by environmental carcinogens. Most cancers are monoclonal in origin (derived from a single cell) and do not arise from a single critical mutation but from the accumulation of sequential critical mutations in relevant target genes within a single cell. Initially a somatic mutation occurs in a critical gene, and this provides a growth advantage to the cell and results in the expansion of the mutant clone. Each additional critical mutation provides a further selective growth advantage resulting in clonal expansion of cells with mutations in multiple critical genes. It often requires decades for a cell clone to accumulate multiple critical mutations and for the progeny of this cell to clonally expand to produce a clinically detectable cancer. Thus the time required for accumulation of mutations in critical genes within a cell is likely related to the observation that cancer incidence increases exponentially with age.

Specific genes found in normal cells, termed proto-oncogenes, are involved in the positive regulation of cell growth and are frequently mutated in cancer. Mutational alteration of these proto-oncogenes can result in a gain of function, for example, the altered gene product can continually stimulate cell proliferation. Proto-oncogenes with gain-of-function mutations are now referred to as oncogenes. Another family of genes, known as tumor suppressor genes can be mutationally inactivated during carcinogenesis resulting in a loss of function. Tumor suppressor genes and the proteins they encode often function as negative regulators of cell growth. Tumor suppressor genes containing loss-of-function mutations encode proteins that are by and large inactive.

Activation of oncogenes and inactivation of tumor suppressor genes within a single cell are important mutational events in carcinogenesis. A simple analogy can be made to the automobile; tumor suppressor genes are analogous to the brakes on the car while the proto-oncogenes are analogous to the accelerator pedal. Mutations within tumor suppressor genes inactivate the braking system while mutations in proto-oncogenes

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activate the acceleration system. In addition to the regulation in cell growth, some oncogenes and tumor suppressor genes can also impair the cells ability to undergo apoptosis or programmed cell death. Mutations in oncogenes and tumor suppressor genes provide a selective growth advantage to the cancer cell through enhanced cell growth and decreased apoptosis.

Cancer is a type of a neoplasm or tumor. While technically a tumor is defined as only a tissue swelling, the term is now used as a synonym for a neoplasm. A neoplasm or tumor is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with the normal tissue, and persists after cessation of the stimuli that evoked it. There are two basic types of neoplasms, termed benign and malignant. Cancer is the general name for a malignant neoplasm. In terms of cancer nomenclature, most adult cancers are carcinomas that are derived from epithelial cells (colon, lung, breast, skin, etc). Sarcomas are derived from mesenchymal tissues, while leukemias and lymphomas are derived from blood-forming cells and lymphoid tissue. Melanoma is derived from melanocytes and retinoblastoma, glioblastoma, and neuroblastoma are derived from the stem cells of the retina, glia, and neurons, respectively. According to the American Cancer Society, (1) the lifetime risk for developing cancer in the United States is about 1 in 3 for women and 1 in 2 for men, (2) in 2003 about 1.3 million new cancer cases are expected to be diagnosed not including carcinoma in situ or basal or squamous cell skin cancer, and (3) cancer is a leading cause of death in the United States and approximately 25% of all deaths are due to cancer.

Human Cancer [41-44]

Although cancer is known to occur in many groups of animals, the primary interest and the focus of most research is in human cancer. Nevertheless, much of the mechanistic research and the hazard assessment are carried out in experimental animals. A consideration of the general aspects of human carcinogenesis follows.

Causes, Incidence, and Mortality Rates of Human Cancer

Breast, lung, and colon and rectum cancers are the major cancers in females while prostate, lung, and colon and rectum are the major cancer sites in males. In addition to lung cancer, smoking also plays a significant role in cancer of the mouth, esophagus, pancreas, pharynx, larynx, bladder, kidney, and uterine cervix. Overall, the age-adjusted national total cancer death rate is increasing. In 1930 the number of cancer deaths per 100,000 people was 143. In 1940, 1950, 1970, 1984, and 1992 the rate had increased to 152, 158, 163, 170, and 172, respectively. According to the American Cancer Society, when lung cancer deaths due to smoking are excluded, the total age-adjusted cancer mortality rate had actually decreased by 16% between 1950 and 1993. However, it is important to realize that death and incidence rates for some types of cancers are increasing while the rates for others are decreasing or remaining constant. Major insights into the etiologies of cancer have been attained through epidemiological studies that relate the roles of hereditary, environmental, and cultural influences on cancer incidence as well as through laboratory studies using rodent/cellular systems. Cancer susceptibility is determined by complex interactions between age, environment, and an individual's genetic makeup. It is estimated from epidemiological studies that 35–80% of all cancers are associated with the environment in which we live and work. The geographic migration of immigrant populations and differences in cancer incidence among communities has provided a great deal of information regarding the role of the environment and specific cancer incidences. a role of the environment in the etiology of cancer. It should be noted that the term environment is not restricted to exposure to human-made chemicals in the environment but applies to all aspects of our lifestyle including smoking, diet, cultural and sexual behavior, occupation, natural and medical radiation, and exposure to substances in air, water, and soil. Only a small percentage of total cancer occurs in individuals with a hereditary mutation/hereditary cancer syndrome (ca. 5%). However, an individual's genetic background is the "stage" in which the cancer develops and susceptibility genes have been identified in humans.

For example, genetic polymorphisms in enzymes responsible for the activation of chemical carcinogens may represent a risk factor as is the case for polymorphisms in the *N*-acetyl-transferase gene and the risk of bladder cancer. These types of genetic risk factors are of low penetrance (low to moderate increased risk); however, increased risk is usually associated with environmental exposure.

Teratogenesis

Developmental toxicity is any morphological or functional alteration caused by chemical or physical insult that interferes with normal growth, homeostasis, development, differentiation, and/or behavior. Teratology is a specialized area of embryology. It is the study of the etiology of abnormal development (the study of birth defects). Teratogens therefore are xenobiotics and other factors that cause malformations in the developing conceptus. Examples of teratogens may include: pharmaceutic compounds, substances of abuse, hormones found in contraceptive agents, cigarette components, and heavy metals. Also included in this category are viral agents, altered metabolic states induced by stress, and nutrient deficiencies (e.g., folic acid deficiency).

Principles of Teratology [39-42]

James Wilson (in 1959) proposed six principles of teratology. A simplified version of these is as follows:

- Susceptibility to teratogenesis depends on the embryo's genotype that interacts with adverse environmental factors (G × E interaction).
- The developmental stage of exposure to the conceptus determines the outcome.
- Teratogenic agents have specific mechanisms through which they exert their pathogenic effects.
- The nature of the teratogenic compound or factor determines its access to the developing conceptus/tissue.
- The four major categories of manifestations of altered development are death, malformation, growth retardation, and functional deficits.
- The manifestations of the altered development increase with increasing dose (i.e. no effect to lethality).

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Guidelines on Handling Chemical Carcinogens, Teratogens and Mutagens [33-38]

Background, Scope and Purpose of the Code

The Control of Substances Hazardous to Health Regulations 1994 introduced specific legal duties relating to the use of carcinogens, including a hierarchy of precautions which are MANDATORY. In addition the Approved Code of Practice on the use of carcinogens was revised and anyone who is responsible for determining departmental policy is strongly advised to obtain a copy. The purpose of this document is to provide Heads of Departments and others responsible for the use of known or suspected carcinogens, teratogens and mutagens guidelines for use. These guidelines can be incorporated, where appropriate, into departmental policies and specific experimental procedures.

Definitions

Carcinogens are agents which cause cancer.
They can be categorised according to the degree of certainty that they cause cancer:

- known carcinogens
- suspected carcinogens
- agents of undetermined carcinogenicity
- non-carcinogens

This document relates to known and suspected carcinogens, although all undetermined agents must be handled with caution [43-45].

Classes of Chemical Carcinogens

The following classes of chemicals are known to contain carcinogens:

- polycyclic aromatic hydrocarbons
- aromatic amines
- N-nitroso compounds
- azo dyes
- alkylating agents
- some naturally occurring compounds

Background Information of Carcinogenesis [24,40-45]

Carcinogenesis is the name given to the process by which cancer develops from normal tissues and their constituent cells. Irrespective of the nature of the causative agents, the process is characterized by certain general properties which are stated here because they illustrate some of the problems encountered in the identification of a carcinogenic hazard.
Cancer is a common condition and is thought to be most often due to environmental causes rather than arising spontaneously. Important known associations with an increased risk of cancer in humans include smoking (lung cancer), sexual promiscuity (cancer of the cervix) and low-fibre diet (large bowel cancer).

Dose

Some carcinogens are extremely potent and can induce cancer at very low dose levels in a susceptible species. There is often no knowledge available about the lower threshold of dose below which cancer will not occur. The probability that cancer will result is usually proportional to the dose, except that very high doses may have more immediate toxic effects.

Duration of Exposure

Unlike radiation protection control, there is no simple way of monitoring individual exposure to chemical carcinogens. A single exposure to a carcinogen may be sufficient to induce cancer.

Latency

With carcinogens, there is no immediate indication that harm has resulted from exposure, unless the agent has some other toxic effect. Long intervals elapse between exposure to carcinogens and the appearance of tumours resulting there from. Intervals of two or three decades are not unusual.

Co-factors

Some carcinogenic agents are unable to produce cancer alone. Subsequent exposure to another agent is necessary to amplify or promote the initial carcinogenic injury.

Routes of Entry

Carcinogens can enter the human body by the following routes:

- by mouth into the gut
- by inhalation into the lungs
- by skin contact
- The resulting cancers do not necessarily appear at the site of entry, because carcinogens require chemical transformation in the body into their active form.

Prohibitions Relating to Certain Substances

The use of the following substances is banned under the Control of Substances Hazardous to Health Regulations 1994:

- 2-naphthylamine
- Benzidine
- 4-aminodiphenyl
- 4-nitrodiphenyl
- The ban includes their salts and any other substance containing more that 0.1% of them.

The use of benzene is also prohibited for most purposes. Although its use in research and analysis is permitted under law, it is the University policy to ban its use unless specific permission for a given procedure has been obtained from Safety Services.

Assessment of Procedures

There is a requirement under CoSHH to assess any procedure involving a hazardous substance before the commencement of work. This is particularly vital with carcinogens since there is unlikely to be any early warning of adverse effects. The general University CoSHH form B should be used as a guide.
The results of the assessment should include at least details of:

- the nature, hazard and extent of potential exposure
- any workers who may be particularly at risk, including the possible risk to pregnant women
- whether substitution by a less hazardous substance is reasonably practicable
- the control measures to be applied
- operating procedures to ensure minimum exposure
- procedures for maintenance and emergencies

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- use of personal protective equipment
- monitoring procedures (if appropriate)
- health surveillance (if appropriate)
- arrangements for information and training
- The assessment should be reviewed:
- if there is any indication that control measures are not working efficiently
- at least every year

Recording of Procedures

All work involving known or suspected carcinogens must be pursued according to written departmental procedures. Specific procedures for each operation will be drawn up in the light of the assessments.

Use of Alternative Substances

Investigators should find out whether suitable, safer alternatives exist before using a reagent which is a known or suspected carcinogen. Obviously if the experiment involves carcinogenesis, then there can be no alternative but to use the material. Safer alternatives may exist if the agent is being used for other laboratory purposes. In chemical synthesis, due consideration should be given to the possible carcinogenic properties of starting materials, intermediates, reaction products and by-products [44-45]. Unfamiliar techniques should be practiced using a non-carcinogen before commencing work with the carcinogen(s) and with due regard for the Home Office regulations in the case of animal work.

CONCLUSION

The Head of Department or person with designated authority must approve all new and existing procedures involving known or suspected carcinogens. They must be satisfied that the use of the agent is essential, that the proposed scale of the work is justified, that adequate facilities exist for use, storage and disposal, and that the investigator in charge of the project is competent to work with the agent. A written record of the project summary, the known or suspected carcinogen, the quantity used, handling and disposal procedure, and the name(s) of the user must be kept in the departmental records for 40 years. If the department ceases to exist, the record must be deposited in a suitable archive. The use of carcinogens, particularly those regulated by law, for teaching purposes should be avoided. If it is considered that their use in a teaching procedure is unavoidable, the need and conditions of use must be reviewed annually by the Head of Department. In any case, all the legally required safety precautions must be followed.

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