Background Information

An Introduction to Toxicology

Toxicology is the science of poisons (also called toxins). It deals with the chemical nature of poisonous substances and how they produce adverse effects in biological systems. A poison is generally defined as any chemical agent that has the capacity to produce abnormal, undesirable, or harmful changes to an organism exposed to it. Poisoning by a chemical agent is equivalent to chemically induced disease. In humans, adverse effects of poisons can range from minor symptoms like headache and nausea to severe ones like convulsions and coma. The ultimate adverse effect is death.

The toxicity of a chemical is an inherent property of the chemical. It cannot be changed without changing the chemical to another form. Most toxins work by altering basic cellular metabolic functions in ways that disrupt normal physiological processes. They are also the target of biochemical reactions in the body that render them inactive (detoxification).

Toxicology is a complex, interdisciplinary subject, straddling the fields of chemistry, biology, pharmacology, medicine, genetics, economics, and law. Contemporary toxicology is usually divided into three main branches:

- clinical toxicology, which deals with the effects of toxins (typically in the form of drugs) on human patients;
- forensic toxicology, which is concerned with detecting the criminal use of toxic agents; and
- environmental toxicology, which studies the effects of industrial and agricultural toxins on human health and the environment.

Regulatory toxicology, with its emphasis on public policy/risk assessment, and occupational toxicology, which concerns toxic exposure in the workplace, are also sometimes considered separate branches of toxicology.

While the subject of toxicology can be quite complex, a basic understanding of its fundamental principles is important if citizens are to make learned decisions about the risks and choices inherent to environmental health issues.

The Fundamental Principle of Toxicology

Early humans recognized basically two types of substances—beneficial ones (water, food, and medicine) and harmful ones (poisons). However, more recent science has shown that virtually every substance is toxic, although the quantity or dose needed to cause specific effects varies considerably. Paracelsus (1493–1541) stated this idea nearly five centuries ago: "All substances are poisons; there is none that is not a poison. The right dose

differentiates a poison and a remedy." For example, two tablets of Extra-Strength Tylenol® (acetaminophen) may relieve a headache, but 40 tablets (20 grams) taken at once can cause serious liver injury or death. Even plain water can be a fatal poison. One German toxicologist reported on a man who died from cerebral edema and severe electrolyte imbalance after drinking 17 liters of water within a very short time. Table 1 shows the probable lethal doses for some common household substances if consumed in one sitting.

Table 1: Probable Oral Lethal Dose for Some Common Substances (150-Pound Human)

water	about 10 quarts
sugar	2.5 pounds
salt	7 ounces
coffee (caffeine)	100 cups
cigarettes (nicotine)*	1 pack
aspirin	100 tablets
80-proof whiskey (ethyl alcohol)	about 1 liter
spinach (oxalic acid)	about 15 pounds

Adapted from: *The Polar Bear Strategy,* 1999, p. 132.
* Assumes 3 mg nicotine/cigarette at lethal oral dose of 60 mg. (See *Clinical Toxicology of Commercial Products,* 1984, p. III-311.)

In terms of toxicity, the general rule is "the dose makes the poison." This concept is often considered the fundamental principal of modern toxicology.

Chronic vs. Acute Exposure

Health effects caused by exposure to toxic substances are usually differentiated based on whether the adverse effect occurs after long-term (chronic) exposure or short-term (acute) exposure. Some substances, such as cyanide, are acutely toxic, which means they cause rapid death after a brief exposure to a lethal (often small) dose. The effects of other substances, such as crocidolite asbestos, are more often cumulative, causing measurable damage only after years of exposure.

How Toxins Work (The Receptor Theory)

All living organisms operate through highly integrated sets of biochemical reactions, which are sensitive to conditions including temperature, pH, and the concentration of other chemicals in the system. Some chemicals, such as strong acids and bases, are toxic simply because they denature proteins and dissolve living tissue. Other chemicals, however, exert their toxic effect by binding with specific receptors in cells, thereby disrupting normal

biochemical reactions. For example, carbon monoxide interferes with human respiration because it has a much stronger affinity for hemoglobin than oxygen does. With the hemoglobin's oxygen receptors tied up, the body cannot process sufficient oxygen, and death may occur.

In multicellular animals, including humans, the body has many finely tuned regulatory systems to ensure things work properly in response to external conditions. This tendency of an organism to maintain normal stability or equilibrium is called homeostasis. Some things can disrupt homeostasis, such as external physical circumstances (extreme heat or cold) or internal chemical or biological agents. For example, organophosphate compounds (including the deadly nerve gas sarin and a common insecticide called parathion) affect homeostasis by deactivating an enzyme responsible for the uptake of the key neurotransmitter acetylcholine. The nervous system becomes overstimulated and induces the sweat glands to produce sweat and the body's muscles to twitch or convulse. It is this loss of homeostasis that produces the symptoms of poisoning or disease.

The Dose-Response Relationship

The reaction of an organism to exposure to a toxic substance is called the response. The observed response can be any measurable physiological change such as nausea, blindness, sterility, birth defects, or death.

In animal studies, the easiest response to measure is the number of deaths in a population of organisms. Because no two individuals of the same species respond to a given dosage identically, the dose-response function is expressed in statistical terms. The comparative potency of different substances is often expressed as "lethal dose fifty" (LD_{50}), the dose at which death is observed in 50 percent of the experimental organisms in question. A similar term, "lethal concentration fifty" (LC_{50}), is used when the toxicants are gases, vapors, or particulates.

The relationship between chemical dose and the health effects observed in a test species are plotted on a dose-response curve. Two general curves are recognized: those in which no response is observed until some minimal dose (threshold) is reached, and those in which even minuscule doses are associated with some degree of risk of adverse effect (nonthreshold).

Figure 1 shows a hypothetical dose-response curve based on a threshold dose-response relationship. Below a given threshold, no observable health effects occur. Above this threshold, effects begin to be seen. Larger and larger doses cause increased adverse effects, until at some level the quantity becomes lethal to all members of the study population. All noncarcinogenic toxins appear to exhibit a threshold dose.

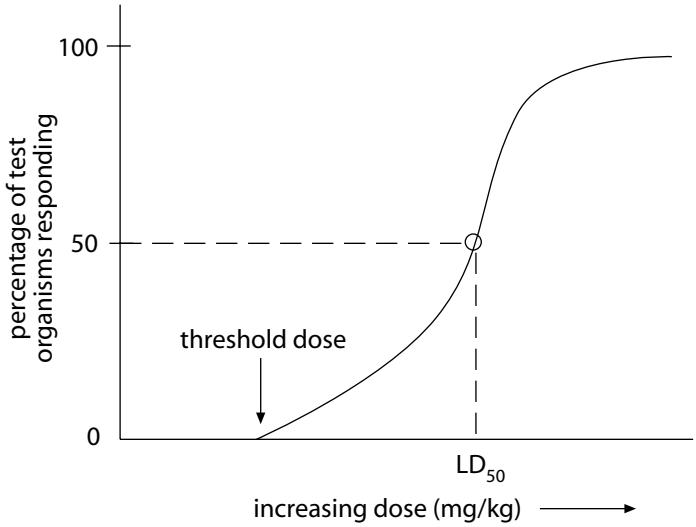

Figure 1: Threshold dose-response curve

The nonthreshold response is based on the assumption that very small doses may cause adverse effects. Because it is impossible to determine effects at extremely low doses, it is assumed that the origin of the dose-response curve is zero and that the shape of the curve is linear. Increases in doses above zero result in increases in adverse effects. At some level, all members of the population will demonstrate the effect under study. The linear (nonthreshold) dose-response curve is shown in Figure 2.

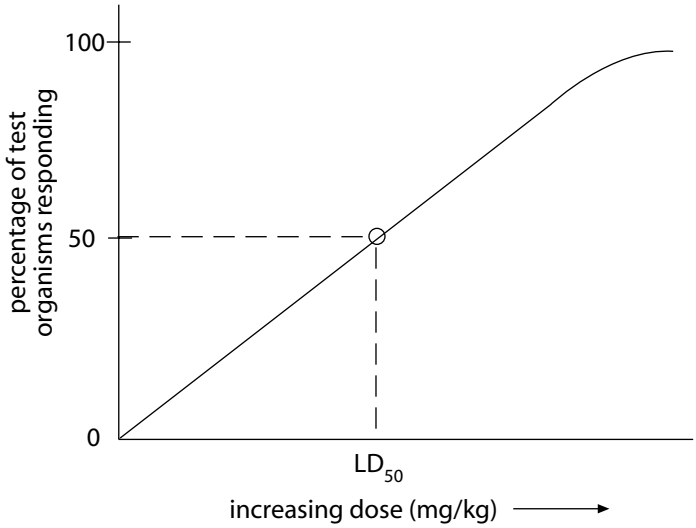

Figure 2: Nonthreshold dose-response curve

Some substances are required in small amounts for normal functioning of an organism yet are toxic at high concentrations. Such substances have a U-shaped (biphasic) dose-response relationship, as shown in Figure 3. Vitamin A, niacin, selenium, and some heavy metal ions are examples of this category.

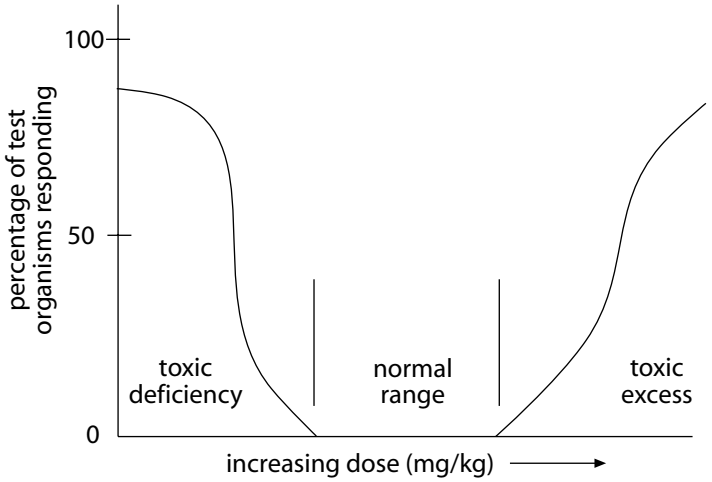

Figure 3: Biphasic dose-response curve

Dose Measurements

The severity and type of a toxin's effect also depends on how rapidly the dose is received (duration) and how often the dose is received (frequency). A chemical may produce no toxic effect if the dose is received slowly enough that the rate of detoxification keeps pace with its intake. The same chemical could produce a toxic effect if received in a rapid dose. Dose, then, is usually specified as the amount (or concentration) of a chemical and the length of time over which it interacts with the organism.

In animal or human studies, the toxic dose of a chemical also usually depends on the size of the organism exposed. Thus, dose is often defined in relative terms rather than absolute quantities. For substances administered orally, dose is measured as quantity per unit mass of the organism and is usually expressed in milligrams per kilogram of body weight (mg/kg). Similarly, chemicals applied to the skin are measured in milligrams per square centimeter (mg/cm^2) of surface area exposed. Inhaled toxins are usually given in concentrations of mass per unit volume or in microliters of vapor or gas per liter of air per volume (ppm). Particulates are usually measured in milligrams per cubic meter of air (mg/m^3).

Comparing the LD_{50} of chemicals provides a relative ranking of toxicity. For example, acrylonitrile (LD_{50} for rats = 78 mg/kg) is considered more toxic than xylene (LD_{50} for rats = 4,300 mg/kg). While LD_{50} is a result of an animal study, the number can also provide a rough estimate of the toxic potential of a chemical in humans. For example, assuming that a rat has a mass of about 1 kg, one would multiply the animal value by 70 kg (average mass of a human) to get an approximate dose that could be expected to kill 50% of humans of average mass.

In environmental or occupational toxicology, dose is usually synonymous with exposure. An important corollary to the idea that all substances are poisons is that virtually any

chemical can be used safely if exposure to the chemical is kept below tolerable limits. Of course, tolerable exposure is extremely low for some highly toxic chemicals. Risk is directly related to the combination of toxicity and exposure. Table 2 shows a common rating system for classifying the toxicity of various substances.

Table 2: Toxicity Rating Definitions

Toxicity Rating	Probable Oral Lethal Dose (Human)	
	Dose	For 70-kg Person
Super Toxic	less than 5 mg/kg	a taste (less than 7 drops)
Extremely Toxic	5–50 mg/kg	between 7 drops and 1 teaspoon
Very Toxic	50–500 mg/kg	between 1 teaspoon and 1 ounce
Moderately Toxic	0.5–5 g/kg	between 1 ounce and 1 pint
Slightly Toxic	5–15 g/kg	between 1 pint and 1 quart
Practically Nontoxic	above 15 g/kg	more than 1 quart
Adapted from *Clinical Toxicology of Commercial Products,* Fifth ed., 1984.		

Limitations of Threshold Dose-Response Data

One limitation of dose-response data is that the LD_{50} value says nothing about toxicity of a chemical at different doses. For example, in Figure 4 chemical A appears to be more toxic than chemical B based on LD_{50}, but at lower doses the chemicals show a reversed toxicity relationship. At LD_{20}, chemical B is more toxic than chemical A.

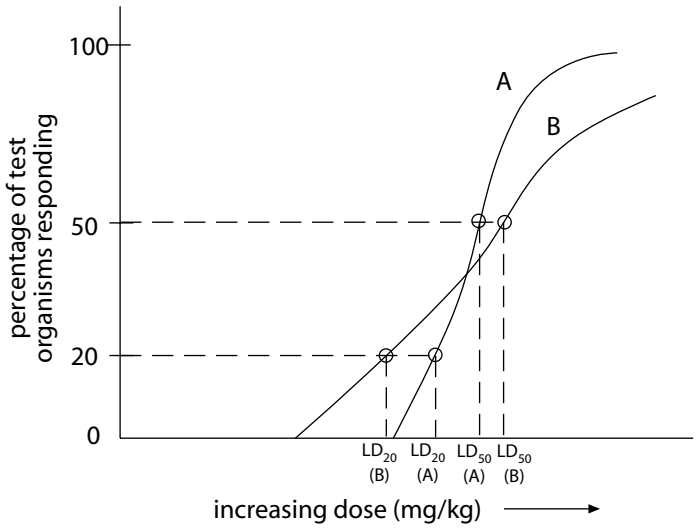

Figure 4: Comparison of dose-response curves for substances A and B

A second problem in using dose-response data is determining which test species most closely approximates the response in humans. A human being is not a 70-kg rat. A dose that is lethal for one species may have little or no effect on another species.

The Cancer Controversy

Although cancer ranks as the second most common cause of death in the United States, carcinogenesis (the production of cancer) is not clearly understood. As a result, evaluating the carcinogenic (cancer producing) potential of chemicals poses several problems. Human health is affected by a wide range of factors, including occupation, genetic disposition, and lifestyle. Therefore, the relationship between any single exposure and the onset of cancer is difficult to determine. Second, many cancers are latent responses—that is, the disease may not show itself until years after the initial exposure.

The concerns of animal-rights activists notwithstanding, animal testing is simply the only way to measure the cancer-causing potential of a chemical when good epidemiological data from human populations is not available. However, animal testing produces its own problems. American regulators usually strive to limit exposure to cancer-causing chemicals to no more than one excess cancer death in a million people. This translates to very tiny doses. Such small exposures are impossible to measure in any practical animal study. The chemical would likely produce no discernible effects in a population of 100 animals (the typical size of such studies). To compensate, investigators often must expose the animal to extremely high doses, usually up to the maximum dose that the animal can tolerate without causing any apparent short-term effect, and extrapolate these data to estimate the risk of low-level human exposure.

There can be obvious drawbacks to this procedure. For example, in 1989 the Natural Resources Defense Council released a report on Alar, a chemical sprayed on apples to promote uniform ripening. The study found that Alar was a potent cancer-causing agent. During the ensuing public health outcry, critics pointed out that experimental rats were fed Alar in concentrations that a human could achieve only by drinking 19,000 quarts of Alar-tainted apple juice per day. Many toxicologists have pointed out that for numerous chemicals, exposure in high doses can kill cells through irritation and trauma alone. These dead cells are replaced through repeated cell divisions that, over time, can initiate cancer. (For further discussion of the Alar scare, see "Toxicology, Risk, and Public Perception: The Alar Controversy" at the end of this Introduction to Toxicology.)

Another difficulty with using animals in cancer studies is the already mentioned problem of interspecies variation. A chemical that causes cancer in one species may have no effect on another. Ideally, a test species should be selected that most closely approximates the physiological processes of humans. However, the effects exhibited by even closely related species may vary greatly for the same dose received under identical conditions. Just a few

examples are that mice contract cancer from exposure to the pesticide dieldrin, but hamsters do not. Rats and mice contract cancer from exposure to 2-acetylaminofluorene, but guinea pigs and lemmings do not. Mice contract cancer from exposure to perchloroethylene, but rats, rabbits, and guinea pigs do not. Rats often develop cancer from aflatoxin B_1, but mice are relatively resistant to it.

To make matters worse, the same carcinogens can affect different organs in different species. For example, benzidine is reported to cause bladder tumors in humans, liver tumors in hamsters, and acoustic tumors in rats.

Another controversy is the decision to assign cancer risk on the assumption that carcinogens have a nonthreshold dose-response curve. According to current regulatory policy, one molecule of a cancer-causing substance may be sufficient to trigger cell mutation and cancer. This model has come under fire by Bruce Ames and Lois Gold at the University of California, who point out that most foods we eat have appreciable levels of naturally occurring carcinogens. Clearly, many carcinogens have some threshold value, but the difficulty of measuring these thresholds leads most regulators to emphasize caution.

The model of a nonthreshold dose response leads to another conservative assumption, the assumed linear nature of a carcinogen's dose-response curve. This is also debatable. For example, imagine a study showing that 1,000 out of 10,000 people are killed by leaping from a height of 10 feet. Working the linear model proportionately, we would expect 500 to perish in a 5-foot jump and 100 in a 1-foot jump. By this extrapolation, five people on average would die just by leaping off the thickness of a doormat. Obviously the relationship between number killed and height jumped from is not linear. Although this example is not necessarily analogous to carcinogen exposure, it nevertheless points out one of the pitfalls of linear extrapolation.

How Toxins Reach You

In order to cause adverse effects, toxins must come into contact with living tissue. Toxic substances can enter the body through oral ingestion, inhalation, or skin absorption. (Intravenous injection is another entry route, but it is not normally significant from the point of view of environmental exposure.) After penetrating the initial cellular barrier (the intestinal mucosa, the lining of the respiratory tract, or the skin), the chemical enters the interstitial fluid that fills the extracellular space of all animals. From this fluid, the substance penetrates the capillaries and enters the bloodstream, which transports it throughout the body.

Oral Ingestion

Some chemicals that enter the mouth in pure form may be absorbed rapidly into the bloodstream. This is the method by which nitroglycerin is administered to heart patients.

However, for most substances, retention time in the mouth or esophagus is too short for much absorption to occur. Once in the stomach, the chemical is mixed with food, acid, and gastric enzymes. These stomach contents can alter the toxicity of a substance by influencing its absorption or modifying its chemical structure.

Most of the absorption of orally-ingested toxins takes place in the small intestines. Chemicals that are insoluble in the gastrointestinal fluids are generally excreted with little harm, unless the chemical is corrosive or irritating. For this reason, metallic mercury swallowed from a broken thermometer is usually not toxic because little of it is absorbed. Soluble chemicals are absorbed through the lining in the gastrointestinal tract and transported through the lymphatic system or through the portal vein. This vein carries compounds directly to the liver, which functions to detoxify the substance before it enters the general circulation.

Inhalation

The inhalation, or respiratory, route is the principal mode of entry for chemicals in the form of vapors, gases, mists, or particulates. Once inhaled, the chemicals are either exhaled or deposited in the respiratory tract.

The human respiratory tract consists of three regions: nasopharyngeal, tracheobronchial, and pulmonary. (See Figure 5.) The nasopharyngeal canal, which extends from the nose to the larynx, is lined with ciliated epithelium and mucous glands that filter out large inhaled particles. The tracheobronchial region consists of the trachea, bronchi, and bronchioles, which together form branched, successively narrower airways between the nasopharyngeal region and the alveoli in the lungs. The pulmonary region includes the basic functional units of the lungs and is the primary location of gas exchange. It consists of small bronchioles and the alveoli, which are in contact with the pulmonary capillaries. The alveoli are basically little bubbles.

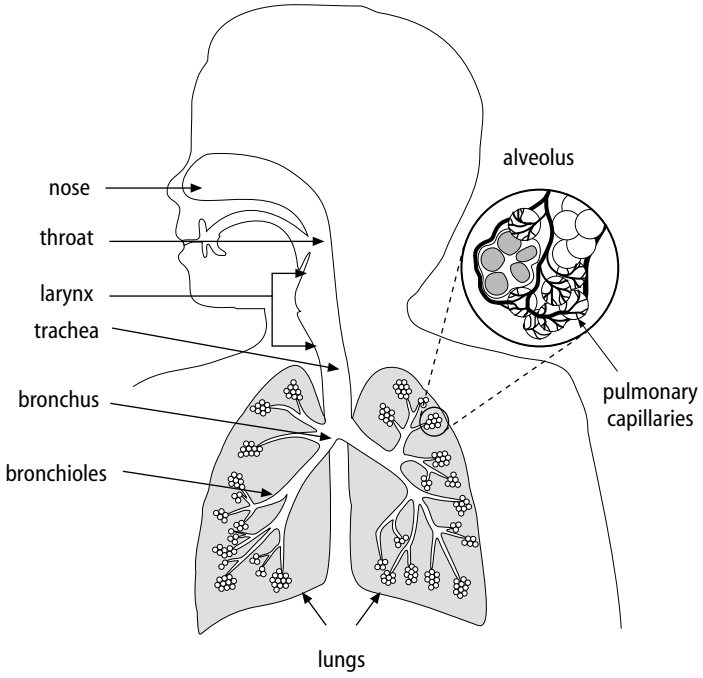

Figure 5: Features of the human respiratory system

Inhaled toxins can cause harm by irritating and destroying respiratory tissue or by being absorbed into the blood. Once in the bloodstream, the substance can be carried to organs that have a special affinity for or sensitivity to the substance's toxic effect.

Chemical substances that can produce acute adverse effects to the respiratory tract include ammonia, chlorine, isocyanates, and phosgene. These substances weaken the capillary walls in the lungs, allowing intravascular fluids to invade the alveoli—a condition known as pulmonary edema, in which victims may literally drown in their own bodily secretions.

Skin Absorption

The skin acts as a protective barrier separating the rest of the body from the environment. The skin consists of three layers: the epidermis, which is the outermost protective layer; a middle layer of vascularized connective tissue called the dermis; and an inner layer consisting of fatty connective tissue called the hypodermis. (See Figure 6.)

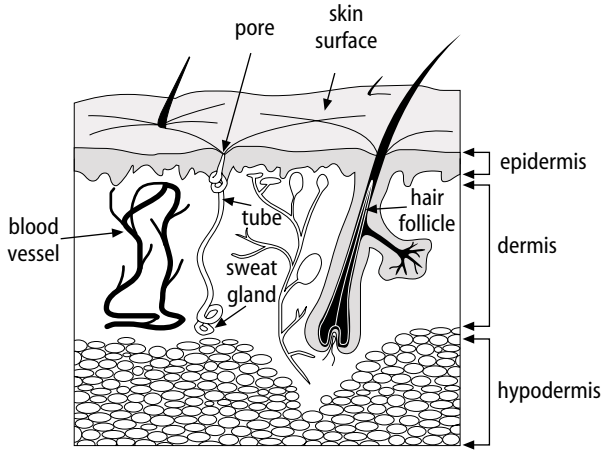

Figure 6: Structure of human skin

Chemicals can penetrate the skin through three mechanisms: diffusion through the epidermis, entry into the sweat ducts, or entry along the hair follicles. Chemicals that can be absorbed through the skin include aniline, hydrogen cyanide, organic mercury compounds, nitrobenzene and phenol. Phenol can be lethal if absorbed for a sufficient time from a few square centimeters of skin.

Toxicokinetics

Toxicokinetics deals with the distribution, metabolism, and elimination of toxins in the body. Once chemicals enter the body, they are metabolized, with some eliminated and some retained. Various elimination mechanisms such as exhalation, perspiration, urination, defecation, or detoxification rid the body of the chemical over a period of time. For some chemicals, the elimination rate is so slow that they may persist in the body for a lifetime. These retained substances may reside in fatty tissue, bones, or organs.

When chemicals are absorbed, they may spread through the bloodstream in unmodified form or be transformed before reaching specific receptors. As previously mentioned, chemicals absorbed in the gastrointestinal tract are carried by the portal vein to the liver, where they may be altered through various metabolic reactions before being released into general circulation. These reactions are frequently called detoxification, but the term can be misleading because the reactions can create metabolites that in some cases are more toxic than the original chemical (bioactivation).

Hydrophilic (very water-soluble) substances in the bloodstream usually do not need to be metabolized, because they can go directly to the kidneys and be excreted as urine, or they can be exhaled through the lungs if they evaporate easily. The tear ducts and sweat glands are also methods of elimination of some substances.

Substances that are more soluble in fat (lipophilic) tend to accumulate in fatty tissues. In the liver, these substances may undergo two phases of detoxification. Monooxygenation reactions introduce reactive groups that are then conjugated with sugars or other hydrophilic compounds. Depending upon other properties of the original compound, these conjugates are then excreted in the urine or in the bile, which empties into the small intestines.

Factors Influencing Toxicity

The specific response that an organism exhibits from a given dose of a toxin varies depending on the combination of other chemicals in the body (synergistic and antagonistic effects), environmental and lifestyle factors, and genetic variants.

Chemical Combinations

Some combinations of chemicals produce effects that differ from those produced by either chemical alone. Synergistic effects occur when a combination of chemicals causes a greater than additive effect. For example, cigarette smoking combined with heavy asbestos exposure results in a risk of lung cancer greater than the sum of each risk alone. Conversely, one chemical can inhibit or diminish the toxicity of another chemical. Some extremely toxic chemicals can be rendered virtually harmless in the presence of an antagonist. This antagonistic effect is widely used in the medical field and is the principle on which antivenins and other poison antidotes work.

Environmental and Genetic Factors

Environmental factors can also contribute to the response for a given toxin. Previous chemical exposure may act in conjunction with other toxic mechanisms. People whose livers have been impaired through alcoholism or other diseases are often more susceptible to the effects of toxic substances than are those with healthy livers.

General factors that contribute to individual variation include age and overall state of health. Infants and children are a special category due to their continued organ development and rate of growth, small size, and relatively high food intake and level of activity. The elderly tend to experience metabolic changes that lower resistance to toxins. Chemicals that affect the reproductive system can have disproportionate effects on a particular gender. In addition to these general factors, everyone possesses their unique genetic makeup that contributes to their particular pattern of disease resistance and susceptibility.

In many cases, an individual's susceptibility to a chemical hazard is due to a combination of both environmental agents and genetically inherited traits. A familiar example is lung cancer. The great majority of people who get lung cancer are smokers, yet we know that many smokers do not get the disease. For this and other diseases, potentially susceptible persons may never get the disease unless they are exposed to environmental agents that serve as the trigger.

Recent advances in genetics provide a new and powerful way to detect these inherited predispositions and to pursue the nature of related environmental effects at a molecular level. In February 2001, scientists reported a new benchmark in science: the list and the DNA sequence of all human genes. As a direct result of that work, progress in identifying what roles the separate genes code for is expanding rapidly. Dr. McCusick at Johns Hopkins University, who has maintained the definitive data on Mendelian inheritance in humans, addressed this in an article announcing the human genome sequence in the journal *Science.* He and his coworkers pointed out that fewer than 50 disease genes had been discovered by the end of 1988, but by the time their manuscript was published, there were 1,100 genes known to be related to over 1,100 recognized genes known to be related to over 1,400 recognized diseases.

Most if not all of those diseases involve the function or malfunction of more than one gene, and also the effects of one or more environmental factors. The new role of toxicology is to relate known and suspected toxic agents to the specific genes and pathways that result in individual susceptibility or resistance. Answers to these questions will allow for less reliance on probability of risk based on the population at large, and for more information on individual risk based on the individual's environment and lifestyle. With this new information will come opportunities for the design of commercial chemicals of decreased toxicity. Toxicology will also contribute to more accurate diagnoses and for more specific drugs and other procedures for disease prevention and treatment.

Toxicology, Risk, and Public Perception: The Alar Controversy

Many of the issues discussed in this Introduction to Toxicology became pertinent to the general public during the Alar scare of 1989. Alar was a growth-regulating chemical used on many fruit trees, most particularly on trees with ripening apples. The result was a crop that was more attractive to the consumer and easier to harvest and process. The apples were used in applesauce and apple juice, staple baby foods.

One of the big problems at the time was that even where some risk estimates were in place, the science was particularly deficient for evaluating the risk to infants and growing children, whose brains and organs are still developing. For this reason, the U.S. EPA is now systematically collecting baseline measures for children and their environment. (For more information, see the December 2000 report "America's Children and the Environment" available at *http://www.epa.gov/children/indicators/.)*

In general, for suspected carcinogens, data are collected by a standard National Institutes of Health-National Cancer Institute (NIH-NCI) protocol unless epidemiological data are available for humans from some horrendous accident or policy (such as massive exposure to benzene or vinyl chloride, or the use of mustard gas in WWI). NIH-NCI protocols involve relatively small numbers of two species of rodents for both sexes, with matched controls, in

lifetime studies. The doses used are chosen based on a preliminary short-term study that uses a range of high doses to establish a measurable impact on the animal. The lifetime study is then conducted at two doses, the highest dose giving no observable short-term effect and one-half of that dose. Then the results are extrapolated to lower doses assuming the relationships are linear and without a threshold. To relate these data to humans, additional numbers are included to be on the safe side in case humans are actually more sensitive than the types of rodents tested. This approach is better than just using the chemical at uncontrolled levels until human cancer shows up.

Research into the Alar controversy will show that the first alarming data were based all or in part on doses that were too high for the established protocol. A number of studies with other chemicals have shown that excessive doses can cause cell-killing, and then replacement of the lost cells allows for an increase in cancer. The scare was received by the public as a crisis-level health threat even though there were few data to use for extrapolation to children. More accurate data should have been collected at accepted doses and analyzed to estimate risk before taking any alarm to the public. However, even now scientists disagree on the strength of all the available data. The biggest difference of opinion generally goes back to the use of the no threshold model vs. a model that assumes humans have sufficiently protective metabolic capacity to detoxify the low levels they may be exposed to.

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