

ENV 108:

Handout Readings

Table of Contents

Manifestations of Toxic Effects	Chapter 1
Entry & Fate of Chemicals in Humans	Chapter 2
How much is a Part Per Million?	Chapter 3
Bioaccumulation	Chapter 4
Dose-Response Relationships in Toxicology	Chapter 5
Carcinogenesis	Chapter 6
Ecological Effects	Chapter 7

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Chapter 1 - MANIFESTATIONS OF TOXIC EFFECTS

INTRODUCTION

The human body is a vastly complex biochemical organism, finely tuned and adaptable. It contains many different regulatory systems to make sure that things work properly in response to external conditions. When it becomes too warm inside the body, the water cooling system is turned up, and more sweat is secreted by the skin. The sweat evaporates, cools the blood underneath the skin, which in turn cools the body core. The sensors in the brain detect that things are back within normal limits, and turn off the sweat glands. This type of regulation (known as homeostasis) occurs for all bodily processes, and usually without any awareness or thought on our part.

When external circumstances (like extreme heat or cold) or internal conditions (disease or poisoning) cannot be adjusted by normal mechanisms, the signs of discomfort and disease appear. The types of physical effects seen or felt (signs and symptoms) depend on the type of stress to which the body has been exposed. Because there are so many complex interrelationships between the systems within the body, a single change in any one system may result in numerous effects in other systems. In addition, the types of response to disease are limited, thus signs and symptoms of disease are often quite similar for different diseases. For example, headache, fever, nausea, vomiting and diarrhea are very common non-specific symptoms of disease, produced by many, many conditions. Because of the generality of most physiological responses to disease, many other methods have been developed to help diagnose the actual causes of disease. These methods include physical, biochemical and immunological techniques upon which modern clinical medicine is based.

A body's homeostasis can be upset by physical, chemical and/or biological agents which put stress on the body. The body's reaction to prolonged stress depends on the nature of the agent, the degree of stress, and the duration of stress. When the stress is too strong or too long, and homeostasis cannot be maintained or restored, disease occurs. Poisoning by chemical agents is nothing more than chemically induced disease, and the symptoms of chemical poisoning often are the same as symptoms caused by biological agents such as bacteria or viruses. To better understand how disease is caused by exposure to toxic chemicals, we must first understand how poisons work within the body.

HOW POISONS WORK

Poisons work by changing the speed of different body functions, increasing them (for example, increasing the heart rate or sweating), or decreasing them (sometimes to the point of stopping them entirely, like breathing). For example, people poisoned by parathion (an insecticide) may experience increased sweating. Increased sweating due to parathion poisoning results as follows. The first step is the biochemical inactivation of an enzyme. This (1) biochemical change leads to a (2) cellular change (in this case an increase in nerve activity). The cellular change is then responsible for (3) physiological changes, which are the symptoms of poisoning that are seen or felt in particular organ systems (in this case the sweat glands). The basic progression of effects from biochemical to cellular to physiological occurs in most all cases of poisoning.

Depending on the specific biochemical mechanism of action, a poison may have very widespread effects throughout the body, or may cause a very limited change in physiological functioning in a particular region or organ. Parathion causes a very simple inactivation of an enzyme that is involved in communication between nerves. The enzyme which parathion inactivates however, is very widespread in the body, and thus many varied effects on many body systems are seen besides sweating.

TOXICITY

Toxicity is a general term used to indicate adverse effects produced by poisons. These adverse effects can range from slight symptoms like headaches or nausea, to severe symptoms like coma and convulsions and death.

Toxicity is normally divided into four types, based on the number of exposures to a poison and the time it takes for toxic symptoms to develop. The two types most often referred to are acute and chronic. Acute toxicity is due to short-term exposure and happens within a relatively short period of time, whereas chronic toxicity is due to long-term exposure and happens over a longer period.

Most toxic effects are reversible and do not cause permanent damage, but complete recovery may take a long time. However, some poisons cause irreversible (permanent) damage. Poisons can affect just one particular organ system or they may produce generalized toxicity by affecting a number of systems. Usually the type of toxicity is subdivided into categories based on the major organ systems affected. Some of these are listed in table 1.

Because the body only has a certain number of responses to chemical and biological stressors, it is a complicated business sorting out the signs and symptoms and determining the actual cause of human disease or illness. In many

cases, it is impossible to determine whether an illness was caused by chemical exposure or by a biological agent (like a flu virus). A history of exposure to a chemical is one important clue in helping to establish the cause of illness, but such a history does not constitute conclusive evidence that the chemical was the cause. To establish this cause/effect relationship, it is important that the chemical be detected in the body (such as in the blood stream), at levels known to cause illness. If the chemical produces a specific and easily detected biochemical effect (like the inhibition of the enzyme acetylcholinesterase), the resulting biochemical change in the body may be used as conclusive evidence.

People who handle chemicals frequently in the course of their jobs and become ill and need medical attention should tell their physicians about their previous exposure to chemicals.

Table 1. General toxicity categories

Category	System affected	Common symptoms
respiratory	nose, trachea, lungs	irritation, coughing, choking, tight chest
gastrointestinal	stomach, intestines	nausea, vomiting, diarrhea
renal	kidney	back pain, urinating more or less than usual
neurological	brain, spinal cord	headache, dizziness, behavior confusion, depression, coma, convulsions
hematological	blood	anemia (tiredness, weakness)
dermatological	skin, eyes	rashes, itching, redness, swelling
reproductive	ovaries, testes, fetus	infertility, miscarriage

Chapter 2 - ENTRY AND FATE OF CHEMICALS IN HUMANS

ROUTES OF ENTRY

Chemicals, including pesticides, are widely distributed in the environment. Therefore there are many possible sources of exposure to these chemicals for humans. Substances which are in ambient and indoor air may be inhaled into the lungs while those in water or food may be ingested or inhaled through mist or steam (such as in the shower). Direct contact with the chemical is the most prevalent way environmental chemicals can penetrate the skin, but exposure through the skin may also occur as a result of contact with chemical contaminants in air and water (for example bathing or swimming).

A single chemical can enter the body through all three routes of exposure -- inhalation, ingestion and skin penetration (dermal exposure). A compound, such as chloroform, which evaporates readily and which may be found in drinking water illustrates this point. When this water is used for drinking, ingestion is the route of exposure. When it is used for showering, exposure may occur due to inhalation of the steam or mist and from direct contact through the skin. Similarly, pesticide use can involve more than one route of exposure if precautions are not taken. A pesticide that is sprayed can be inhaled during use; penetrate through the skin during mixing and application; and be ingested through food if not washed off hands or food before eating.

ABSORPTION, DISTRIBUTION AND FATE

Once a chemical enters the body, it is often absorbed into the bloodstream and can move throughout the body. The amount absorbed and the rate of absorption depend on the chemical and the route of exposure. This movement of the substance through the bloodstream is called distribution. Through distribution a chemical can come into contact with all parts of the body, not only the original site of entry. In some cases, such contact, distant from the site of entry, can lead to adverse health effects. For example, ingestion of the pesticide parathion into the stomach can lead to damage to the lungs.

Once a chemical is absorbed into the bloodstream, it can have several different fates. In many cases, it is rapidly removed from the body through the urine or feces. In other situations, it may be stored in various parts of the body, such as fat or bone, and remain in the individual for many years. A compound may also lead to a toxic effect through interaction with certain organs or tissues in the individual or with other compounds in the body.

Often, a substance that is absorbed into the body interacts with particular body chemicals and is changed into one or more other chemicals. This process is called metabolism and the products are called metabolites. Metabolism may lead to products that are easier for the body to excrete and so can protect the body from possible adverse effects. In other cases, however, the metabolites may be more toxic than the original chemical which was absorbed. The variety of products resulting from metabolism may have the same possible fates as the original chemical -- storage, excretion or toxicity.

CHEMICAL PROPERTIES

The particular properties of the absorbed chemical are quite critical to its fate in the body. Certain chemicals are very resistant to metabolism and readily dissolve in fat so that they tend to be stored. Dieldrin is a good example of this type of compound. Other chemicals are more rapidly metabolized and excreted and are gone before they can cause adverse effects. The organophosphate pesticides tend to behave this way at low doses.

AN INDIVIDUAL'S CHARACTERISTICS

The characteristics of the individual who is exposed are also very important in the fate of the chemical. The age, sex, genetic background, previous exposures, diet and other factors play important roles in the way that the body interacts with a chemical and in turn the potential for adverse effects. Thus, the characteristics of both the chemical and the exposed individual are important factors determining the fate of the chemical in the body.

THE TIME COURSE FOR EXPOSURE

In the case of a single event exposure, it is the total amount of chemical to which a person is exposed that determines the severity of the toxic effect, if any. The greater the amount of exposure, the greater the potential for adverse health effects. In some cases, this is due solely to the inherent toxicity of the chemical and, in others, also to the overwhelming of the body's ability to respond. In the latter case, the body may not be able to metabolize the chemical rapidly enough to prevent an increase in concentration to toxic levels. In such a situation, there is a clear threshold above which toxic signs and symptoms appear.

In the case of (repeated) multiple exposures to a chemical, it is not only the total amount of exposure, but also the rate or timing of exposure that is quite important. All processes in the body normally proceed at specific rates so that metabolism, excretion and storage occur during a particular period of time after a chemical is absorbed. For a one occurrence exposure, the time needed for the various processes that breakdown the compound to be completed will determine the length of time that a toxic response, if any, persists.

However, if there are repeated exposures to the same chemical, the situation is more complicated. If there is enough time between exposures so that all of the chemical from the initial exposure is excreted, and no effects persist, then each exposure is essentially independent of the previous one and can be treated as a single exposure. However, if the time between exposures is so short that some of the chemical remains from the first exposure, then a buildup of the chemical can occur. Over time this buildup can lead to levels which are toxic.

The total amount of exposure can have different results depending on whether the exposure occurred all at once or repeatedly over time (the time course of exposure). A high dose given once may have a toxic effect while the same total dose given in small amounts over time will not. For example, drinking several ounces of alcohol at once may cause inebriation while drinking one ounce every few hours may not. Also, a particular dose given a few hours apart may have an adverse effect while the same total dose given a few days apart will not.

SUMMARY

The possible toxic effects of exposure to a particular chemical depend on many factors. These include the characteristics of the chemical and the individual exposed; the route of exposure; the total dose and the time course of exposure. Unfortunately, scientists have not been able to determine exactly how each of these factors will affect any specific individual so that present understanding of chemical exposures provides only general guidance. Minimizing exposure will minimize the potential for adverse effects. In addition, a general knowledge of all the contributing factors will help reveal the situations which have the most potential for adverse health effects and can aid in determining the best ways to manage chemicals.

Chapter 3 - HOW MUCH IS A PART PER MILLION?

INTRODUCTION

The health effects of any toxic substance are related to the amount of exposure, also known as the dose. The greater the dose the more severe the effects. Some chemicals can cause toxicity at very low doses and so it is important to be able to understand how these very small amounts are described. It is especially important to understand how low doses compare to one another and what they represent when compared to amounts of more familiar substances.

Parts per million (ppm), parts per billion (ppb), and parts per trillion (ppt), are the most commonly used terms to describe very small amounts of contaminants in our environment. But what do these terms represent? They are measures of concentration, the amount of one material in a larger amount of another material; for example, the weight of a toxic chemical in a certain weight of food. They are expressed as concentrations rather than total amounts so we can easily compare a variety of different environmental situations. For example, scientists can measure the concentration of a chemical in the Great Lakes by looking at small samples. They do not have to measure the total amount of chemicals or water in all of the lakes.

An example might help illustrate the part per ... idea. If you divide a pie equally into 10 pieces, then each piece would be a part per ten; for example, one-tenth of the total pie. If, instead, you cut this pie into a million pieces, then each piece would be very small and would represent a millionth of the total pie or one part per million of the original pie. If you cut each of these million minute pieces into a thousand little pieces, then each of these new pieces would be one part per billion of the original pie. To give you an idea of how little this would be, a pinch of salt in ten tons of potato chips is also one part (salt) per billion parts (chips).

In this example, the pieces of the pie were made up of the same material as the whole. However, if there was a contaminant in the pie at a level of one part per billion, one of these invisible pieces of pie would be made up of the contaminant and the other 999,999,999 pieces would be pure pie. Similarly, one part per billion of an impurity in water represents a tiny fraction of the total amount of water. One part per billion is the equivalent of one drop of impurity in 500 barrels of water.

COMPARISONS AND CONVERSIONS

Sometimes, instead of using the part per ... terminology, concentrations are reported in weight units; such as the weight of the impurity compared to the weight of the total. The metric system is the most convenient way to express this since metric units go by steps of ten, hundred and thousand. For example, a milligram is a thousandth of a gram and a gram is a thousandth of a kilogram. Thus, a milligram is a thousandth of a thousandth, or a millionth of a kilogram. A milligram is one part per million of a kilogram thus, one part per million (ppm) is the same as one milligram per kilogram. Just as part per million is abbreviated as ppm, a milligram per kilogram has its own abbreviation -- mg/kg. Using our abbreviations, one ppm equals one mg/kg.

Kilograms and milligrams are units of weight so they don't apply to volumes of liquids or gases. Instead of a kilogram, the unit of liquid volume most commonly used is the liter. A liter of water weighs one kilogram. If the contaminant is a solid, it is measured in milligrams. Thus, one part per million of a solid in a liquid can be written as a milligram per liter and abbreviated mg/l.

These are the most common units that are encountered. However, with the ability to detect even smaller amounts of contaminants, the terms part per billion and part per trillion are becoming more common. In the metric weight system, a microgram is a thousandth of a milligram. Since a milligram is a millionth of a kilogram, and the microgram is a thousand times smaller, it is equivalent to a billionth of a kilogram. Microgram is abbreviated ug. Thus, a part per billion of solid measure is equal to a ug/kg. Similarly, a part per billion of a solid in a liquid is equal to a ug/l.

Before going on to discuss a real example of how these measurements are used, we can compare metric weight quantities to the quantities we are most accustomed to using. A kilogram is equal to about two pounds. Thus, a milligram is less than a millionth of a pound. Looked at another way, it would take about five thousand milligrams (5000 mg) to make up one teaspoonful of a solid (such as salt). The unit of liquid volume, the liter, is very close to a quart. Thus, a milligram per liter is about the same as a milligram per quart.

THE CASE OF PCBs:

An Example

In order to appreciate how these quantities can be used in a real situation, an example is in order. In this example, we use the part per ... terminology to compare the relative importance of PCBs in Great Lakes fish versus

PCBs in Great Lakes drinking water; that is, which source might contribute most to PCB exposure of humans living in the Great Lakes states. The maximum level of PCBs legally allowed in fish sold in interstate commerce is 2 ppm (parts per million). Although there are no legally established levels for PCBs in drinking water, measurements have shown that the average PCB content of the Great Lakes drinking water is about 4 ppt (parts per trillion).

Since a part per trillion is a million times less than one part per million, the maximum allowable concentration of PCBs in fish is about a million times higher than the level of PCBs in drinking water. However, we generally consume a lot more water than fish. At the extreme, people might eat as much as a pound of fish a day or as little as one pound every 100 days (1/100 lb/day). On the other hand, people generally drink about 2 liters (equivalent to about 5 pounds) of water a day.

Thus, the consumption of water might range from about 5 to 500 times the consumption of fish. However, since there are a million times more PCBs in a pound of fish compared to a pound of water, fish can be a much greater source of PCBs than drinking water. The total amount of PCBs consumed depends most on the amount of fish eaten, how contaminated it is, and how it is prepared. Thus, the best way to reduce human exposure to PCBs is to reduce the levels in fish, reduce human consumption of fish with the highest contaminant levels and prepare the consumed fish in the most appropriate manner.

CONCLUSION

The ability to measure concentrations of chemicals in a uniform manner provides a powerful tool for the comparison of water quality from area to area, for the establishment of water quality guidelines or a comparison of doses of chemicals as are commonly found throughout the Pesticide Information Profiles. The use of the metric system provides an easy way to utilize both liquid and solid measurements.

METRIC SYSTEM QUANTITIES

For Solids

1 kilogram (kg) = 1 million milligrams (mg)

so: 1 mg/kg = 1 part per million

1 kilogram (kg) = 1 billion micrograms (ug)

so: 1 ug/kg = 1 part per billion

For Liquids

1 liter (l) of water weighs exactly 1 kg

so: 1 mg/l = 1 part per million and

1 ug/l = 1 part per billion

1 kg = about 2.2 pounds

1 l = about 1 quart

Chapter 4 – BIOACCUMULATION

DEFINING BIOACCUMULATION

An important process through which chemicals can affect living organisms is bioaccumulation. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Understanding the dynamic process of bioaccumulation is very important in protecting human beings and other organisms from the adverse effects of chemical exposure, and it has become a critical consideration in the regulation of chemicals.

A number of terms are used in conjunction with bioaccumulation. Uptake describes the entrance of a chemical into an organism -- such as by breathing, swallowing, or absorbing it through the skin -- without regard to its subsequent storage, metabolism, and excretion by that organism.

Storage, a term sometimes confused with bioaccumulation, means the temporary deposit of a chemical in body tissue or in an organ. Storage is just one facet of chemical bioaccumulation. (The term also applies to other natural processes, such as the storage of fat in hibernating animals or the storage of starch in seeds.)

Bioconcentration is the specific bioaccumulation process by which the concentration of a chemical in an organism becomes higher than its concentration in the air or water around the organism. Although the process is the same for both natural and manmade chemicals, the term bio-concentration usually refers to chemicals foreign to the organism. For fish and other aquatic animals, bioconcentration after uptake through the gills (or sometimes the skin) is usually the most important bioaccumulation process.

Biomagnification describes a process that results in the accumulation of a chemical in an organism at higher concentrations than are found in its food. It occurs when a chemical becomes more and more concentrated as it moves up through a food chain -- the dietary linkages between single-celled plants and increasingly larger animal species.

A typical food chain includes algae eaten by the water flea eaten by a minnow eaten by a trout and finally consumed by an osprey (or human being). If each step results in increased bioaccumulation, that is, Biomagnification, then an animal at the top of the food chain, through its regular diet, may accumulate a much greater concentration of chemical than was present in organisms lower in the food chain.

Biomagnification is illustrated by a study of DDT that showed that where soil levels were 10 parts per million (ppm), DDT reached a concentration of 141 ppm in earthworms and 444 ppm in robins. Through biomagnification, the concentration of a chemical in the animal at the top of the food chain may be high enough to cause death or adverse effects on behavior, reproduction, or disease resistance and thus endanger that species, even when levels in the water, air, or soil are low. Fortunately, bioaccumulation does not always result in biomagnification.

THE BIOACCUMULATION PROCESS

Bioaccumulation is a normal and essential process for the growth and nurturing of organisms. All animals, including humans, daily bioaccumulate many vital nutrients, such as vitamins A, D and K, trace minerals, and essential fats and amino acids. What concerns toxicologists is the bioaccumulation of substances to levels in the body that can cause harm. Because bioaccumulation is the net result of the interaction of uptake, storage and elimination of a chemical, these parts of the process will be examined further.

UPTAKE

Bioaccumulation begins when a chemical passes from the environment into an organism's cells. Uptake is a complex process that is still not fully understood. Scientists have learned that chemicals tend to move, or diffuse, passively from a place of high concentration to one of low concentration. The force or pressure for diffusion is called the chemical potential, and it works to move a chemical from outside to inside an organism.

A number of factors may increase the chemical potential of certain substances. For example, some chemicals do not mix well with water. They are called lipophilic, meaning "fat loving," or hydrophobic, meaning "water hating." In either case, they tend to move out of water and enter the cells of an organism, where there are lipophilic microenvironments.

STORAGE

The same factors affecting the uptake of a chemical continue to operate inside an organism, hindering a chemical's return to the outer environment. Some chemicals are attracted to certain sites, and by binding to proteins

or dissolving in fats, they are temporarily stored. If uptake slows or is not continued, or if the chemical is not very tightly bound in the cell, the body can eventually eliminate the chemical.

One factor important in uptake and storage is water solubility; the ability of a chemical to dissolve in water. Usually, compounds that are highly water soluble have a low potential to bioaccumulate and do not leave water readily to enter the cells of an organism. Once inside, they are easily removed unless the cells have a specific mechanism for retaining them.

Heavy metals like mercury and certain other water-soluble chemicals are such an exception, because they bind tightly to specific sites within the body. When binding occurs, even highly water-soluble chemicals can accumulate. This is illustrated by cobalt, which binds very tightly and specifically to sites in the liver and accumulates there despite its water solubility. Similar accumulation processes occur for mercury, copper, cadmium, and lead.

Many fat-loving (lipophilic) chemicals pass into organism's cells through the fatty layer of cell membranes more easily than water-soluble chemicals. Once inside the organism, these chemicals may move through numerous membranes until they are stored in fatty tissues and begin to accumulate.

The storage of toxic chemicals in fat reserves serves to detoxify the chemical, or at least removes it from harm's way. However, when fat reserves are called upon to provide energy for an organism the materials stored in the fat may be remobilized within the organism and may again be potentially toxic. If appreciable amounts of a toxin are stored in fat and fat reserves are quickly used, significant toxic effects may be seen from the remobilization of the chemical.

ELIMINATION

Another factor affecting bioaccumulation is whether an organism can break down and/or excrete a chemical. The biological breakdown of chemicals is termed metabolism. This ability varies among individual organisms and species and also depends on characteristics of the chemical itself.

Chemicals that dissolve readily in fat but not in water tend to be more slowly eliminated by the body and thus have a greater potential to accumulate. Many metabolic reactions change a chemical into more water-soluble forms called metabolites, that are readily excreted.

There are exceptions, however. Natural pyrethrins, insecticides that are derived from the chrysanthemum plant, are highly fat-soluble pesticides, but they are easily degraded and do not accumulate. The insecticide chlorpyrifos, which is less fat-soluble but more poorly degraded, tends to bioaccumulate. Factors affecting metabolism often determine whether a chemical achieves its bioaccumulation potential in a given organism.

BIOACCUMULATION: A STATE OF DYNAMIC EQUILIBRIUM

When a chemical enters the cells of an organism, it is distributed and then excreted, stored or metabolized. Excretion, storage, and metabolism decrease the concentration of the chemical inside the organism, increasing the potential of the chemical in the outer environment to move into the organism. During constant environmental exposure to a chemical, the amount of a chemical accumulated inside the organism, and the amount leaving, reach a state of dynamic equilibrium.

To understand this concept of dynamic equilibrium, imagine a tub filling with water from a faucet at the top and draining out through a pipe of smaller size at the bottom. When the water level in the tub is low, little pressure is exerted on the outflow at the bottom of the tub. As the water level rises, the pressure on the outflow increases. Eventually, the amount of the water flowing out will equal the amount flowing in, and the level of the tub will not change. If the input or outflow is changed, the water in the tub adjusts to a different level.

It is the same concept with living organisms. An environmental chemical will at first move into an organism more rapidly than it is stored, degraded, and excreted. With constant exposure, its concentration inside the organism gradually increases. Eventually, the concentration of the chemical inside the organism will reach an equilibrium with the concentration of the chemical outside the organism, and the amount of chemical entering the organism will be the same as the amount leaving. Although the amount inside the organism remains constant, the chemical continues to be taken up, stored, degraded, and excreted.

If the environmental concentration of the chemical increases, the amount inside the organism will increase until it reaches a new equilibrium. Exposure to large amounts of a chemical for a long period of time, however, may overwhelm the equilibrium (for example, overflowing the tub) potentially causing harmful effects.

Likewise, if the concentration in the environment decreases, the amount inside the organism will also decline. Should the organism move to a clean environment, so that exposure ceases, then the chemical eventually will be eliminated from the body.

FACTORS AFFECTING BIOACCUMULATION

This simplified explanation does not take into account all of the many factors that affect the ability of chemicals to be bioaccumulated. Some chemicals bind to specific sites in the body, prolonging their stay, whereas others move freely in and out. The time between uptake and eventual elimination of a chemical directly affects bioaccumulation. Chemicals that are immediately eliminated, for example, do not bioaccumulate.

Similarly, the duration of exposure is also a factor in bioaccumulation. Most exposures to chemicals in the environment vary continually in concentration and duration, sometimes including periods of no exposure. In these cases, an equilibrium is never achieved and the accumulation is less than expected.

Bioaccumulation varies between individual organisms as well as between species. Large, fat, long-lived individuals or species with low rates of metabolism or excretion of a chemical will bioaccumulate more than small, thin, short-lived organisms. Thus, an old lake trout may bioaccumulate much more than a young bluegill in the same lake.

SUMMARY

Bioaccumulation results from a dynamic equilibrium between exposure from the outside environment and uptake, excretion, storage, and degradation within an organism. The extent of bioaccumulation depends on the concentration of a chemical in the environment, the amount of chemical coming into an organism from the diet, water, or air, and the time it takes for the organism to acquire the chemical and then excrete, store, and/or degrade it. The nature of the chemical itself, such as its solubility in water and fat, affects its uptake and storage. Equally important is the ability of the organism to degrade and excrete a particular chemical. When exposure ceases, the body gradually metabolizes and excretes the chemical.

Bioaccumulation is a normal process that can result in injury to an organism only when the equilibrium between exposure and bioaccumulation is overwhelmed, relative to the harmfulness of the chemical. Sometimes bioaccumulation can be a protective mechanism in which the body accumulates needed chemicals.

Chapter 5 - DOSE-RESPONSE RELATIONSHIPS IN TOXICOLOGY

"The right dose differentiates a poison and a remedy."

Paracelsus

INTRODUCTION

The science of toxicology is based on the principle that there is a relationship between a toxic reaction (the response) and the amount of poison received (the dose). An important assumption in this relationship is that there is almost always a dose below which no response occurs or can be measured. A second assumption is that once a maximum response is reached any further increases in the dose will not result in any increased effect.

One particular instance in which this dose-response relationship does not hold true, is in regard to true allergic reactions. Allergic reactions are special kinds of changes in the immune system; they are not really toxic responses. The difference between allergies and toxic reactions is that a toxic effect is directly the result of the toxic chemical acting on cells. Allergic responses are the result of a chemical stimulating the body to release natural chemicals which are in turn directly responsible for the effects seen. Thus, in an allergic reaction, the chemical acts merely as a trigger, not as the bullet.

For all other types of toxicity, knowing the dose-response relationship is a necessary part of understanding the cause and effect relationship between chemical exposure and illness. As Paracelsus once wrote, "The right dose differentiates a poison from a remedy." Keep in mind that the toxicity of a chemical is an inherent quality of the chemical and cannot be changed without changing the chemical to another form. The toxic effects on an organism are related to the amount of exposure.

MEASURES OF EXPOSURE

Exposure to poisons can be intentional or unintentional. The effects of exposure to poisons vary with the amount of exposure, which is another way of saying "the dose." Usually when we think of dose, we think in terms of taking one vitamin capsule a day or two aspirin every four hours, or something like that. Contamination of food or water with chemicals can also provide doses of chemicals each time we eat or drink. Some commonly used measures for expressing levels of contaminants are listed in table 1. These measures tell us how much of the chemical is in food, water or air. The amount we eat, drink, or breathe determines the actual dose we receive.

Concentrations of chemicals in the environment are most commonly expressed as ppm and ppb. Government tolerance limits for various poisons usually use these abbreviations. Remember that these are extremely small quantities. For example, if you put one teaspoon of salt in two gallons of water the resulting salt concentration would be approximately 1,000 ppm and it would not even taste salty!

Table 1. Measurements for Expressing Levels of Contaminants in Food and Water.

Dose	Abbrev.	Metric equivalent	Approx. Amount in water
parts per million	ppm	mg/kg: milligrams per kilogram	1 teaspoon per 1,000 gallons
parts per billion	ppb	ug/kg: micrograms per kilogram	1 teaspoon per 1,000,000 gallons

DOSE-EFFECT RELATIONSHIPS

The dose of a poison is going to determine the degree of effect it produces. The following example illustrates this principle. Suppose ten goldfish are in a ten-gallon tank and we add one ounce of 100-proof whiskey to the water every five minutes until all the fish get drunk and swim upside down. Probably none would swim upside down after the first two or three shots. After four or five, a very sensitive fish might. After six or eight shots

another one or two might. With a dose of ten shots, five of the ten fish might be swimming upside down. After fifteen shots, there might be only one fish swimming properly and it too would turn over after seventeen or eighteen shots.

The effect measured in this example is swimming upside down. Individual sensitivity to alcohol varies, as does individual sensitivity to other poisons. There is a dose level at which none of the fish swim upside down (no observed effect). There is also a dose level at which all of the fish swim upside down. The dose level at which 50 percent of the fish have turned over is known as the ED₅₀, which means effective dose for 50 percent of the fish tested. The ED₅₀ of any poison varies depending on the effect measured. In general, the less severe the effect measured, the lower the ED₅₀ for that particular effect. Obviously poisons are not tested in humans in such a fashion. Instead, animals are used to predict the toxicity that may occur in humans.

One of the more commonly used measures of toxicity is the LD₅₀. The LD₅₀ (the lethal dose for 50 percent of the animals tested) of a poison is usually expressed in milligrams of chemical per kilogram of body weight (mg/kg). A chemical with a small LD₅₀ (like 5 mg/kg) is very highly toxic. A chemical with a large LD₅₀ (1,000 to 5,000 mg/kg) is practically non-toxic. The LD₅₀ says nothing about non-lethal toxic effects though. A chemical may have a large LD₅₀, but may produce illness at very small exposure levels. It is incorrect to say that chemicals with small LD₅₀s are more dangerous than chemicals with large LD₅₀s, they are simply more toxic. The danger, or risk of adverse effect of chemicals, is mostly determined by how they are used, not by the inherent toxicity of the chemical itself.

The LD₅₀'s of different poisons may be easily compared; however, it is always necessary to know which animal species was used for the tests and how the poison was administered (the route of exposure), since the LD₅₀ of a poison may vary considerably based on the species of animal and the way exposure occurs. Some poisons may be extremely toxic if swallowed (oral exposure) and not very toxic at all if splashed on the skin (dermal exposure). If the oral LD₅₀ of a poison were 10 mg/kg, 50 percent of the animals who swallowed 10 mg/kg would be expected to die and 50 percent to live. The LD₅₀ is determined mathematically, and in actual tests using the LD₅₀, it would be unusual to get an exact 50% response. One test might produce 30% mortality and another might produce 70% mortality. Averaged out over many tests, the numbers would approach 50%, if the original LD₅₀ determination was valid.

The potency of a poison is a measure of its strength compared to other poisons. The more potent the poison, the less it takes to kill; the less potent the poison, the more it takes to kill. The potencies of poisons are often compared using signal words or categories as shown in the example in table 2.

The designation toxic dose (TD) is used to indicate the dose (exposure) that will produce signs of toxicity in a certain percentage of animals. The TD₅₀ is the toxic dose for 50 percent of the animals tested. The larger the TD the more poison it takes to produce signs of toxicity. The toxic dose does not give any information about the lethal dose because toxic effects (for example, nausea and vomiting) may not be directly related to the way that the chemical causes death. The toxicity of a chemical is an inherent property of the chemical itself. It is also true that chemicals can cause different types of toxic effects, at different dose levels, depending on the animal species tested. For this reason, when using the toxic dose designation it is useful to precisely define the type of toxicity measured, the animal species tested, and the dose and route of administration.

Table 2. Toxicity Rating Scale and Labeling Requirements for Pesticides.

Category	Signal word	Required on label	LD ₅₀ Oral mg/kg (ppm)	LD ₅₀ Dermal mg/kg (ppm)	Probable oral lethal dose
I	Highly toxic	Danger-Poison (skull & crossbones)	Less than 50	Less than 200	A few teaspoons
II	Moderately toxic	Warning	51 to 500	200 to 2,000	Over 1 teaspoon to 1 ounce
III	Slightly toxic	Caution	Over 500	Over 2,000	Over 1 ounce
IV	Practically non-toxic	none required	—	—	—

Toxicity assessment is quite complex, many factors can affect the results of toxicity tests. Some of these factors include variables like temperature, food, light, and stressful environmental conditions. Other factors related to the animal itself include age, sex, health, and hormonal status.

The NOEL (no observable effect level) is the highest dose or exposure level of a poison that produces no noticeable toxic effect on animals. From our previous fish example, we know that there is a dose below which no effect is seen. In toxicology, residue tolerance levels of poisons that are permitted in food or in drinking water, for instance, are usually set from 100 to 1,000 times less than the NOEL to provide a wide margin of safety for humans.

The TLV (threshold limit value) for a chemical is the airborne concentration of the chemical (expressed in ppm) that produces no adverse effects in workers exposed for eight hours per day five days per week. The TLV is usually set to prevent minor toxic effects like skin or eye irritation.

Very often people compare poisons based on their LD_{50} 's and base decisions about the safety of a chemical based on this number. This is an over-simplified approach to comparing chemicals because the LD_{50} is simply one point on the dose-response curve that reflects the potential of the compound to cause death. What is more important in assessing chemical safety is the threshold dose, and the slope of the dose-response curve, which shows how fast the response increases as the dose increases. Figure 1 shows examples of dose-response curves for two different chemicals that have the same LD_{50} . Which of these chemicals is more toxic? Answer this question for doses below the LD_{50} and it is chemical A which is more toxic, at the LD_{50} they are the same, and above the LD_{50} , chemical B is more toxic. While the LD_{50} can provide some useful information, it is of limited value in risk assessment because the LD_{50} only reflects information about the lethal effects of the chemical. It is quite possible that a chemical will produce a very undesirable toxic effect (such as reproductive toxicity or birth defects) at doses which cause no deaths at all.

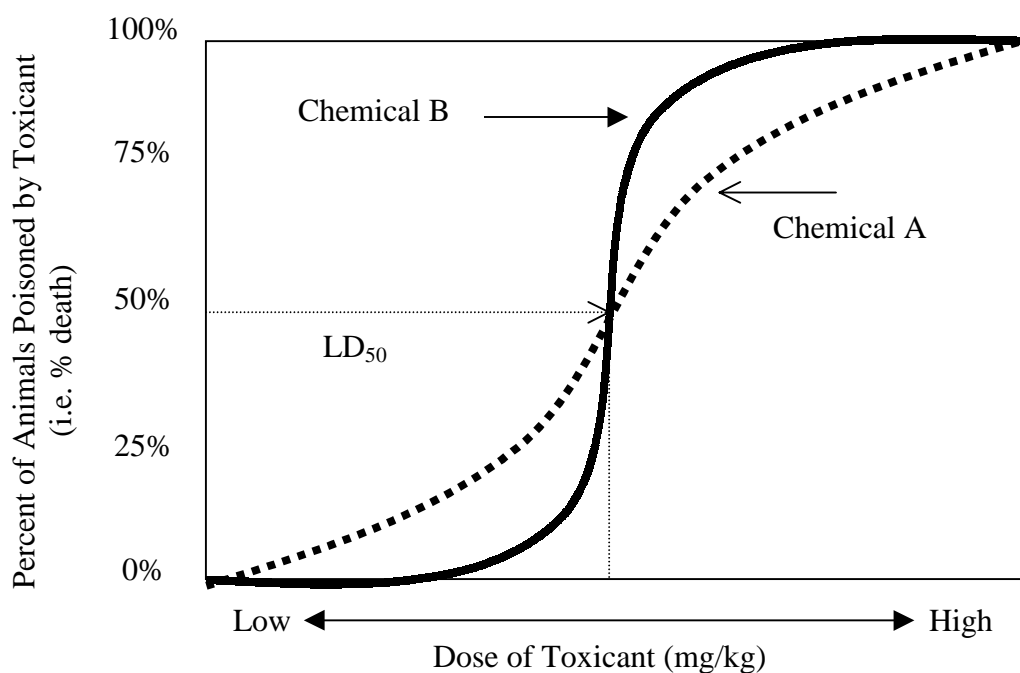


Figure 1: Dose-Response Curves for 2 Chemicals with Different Toxicities

A true assessment of chemical toxicity involves comparisons of numerous dose-response curves covering many different types of toxic effects. The determination of which pesticides will be Restricted Use Pesticides involves this approach. Some Restricted Use Pesticides have very large LD_{50} 's (low acute oral toxicity), however, they may be very strong skin or eye irritants and thus require special handling.

The knowledge gained from dose-response studies in animals is used to set standards for human exposure and the amount of chemical residue that is allowed in the environment. As mentioned previously, numerous dose-response relationships must be determined, in many different species. Without this information, it is impossible to accurately predict the health risks associated with chemical exposure. With adequate information, we can make informed decisions about chemical exposure and work to minimize the risk to human health and the environment.

Chapter 6 – CARCINOGENICITY

INTRODUCTION

Although both natural and synthetic chemicals may cause a variety of toxic effects at high enough doses, the effect that is of most concern in the U.S. is cancer. This is not surprising considering the high incidence of this disease, its often fatal outcome and the overall cost to society. Unfortunately, the incidence of this disease seems to increase with age so that as people live longer, there will be more and more cases of cancer in our country.

Scientists do not yet understand exactly how cancer occurs or why some chemicals seem to cause cancer and others do not. Chemicals that are known to cause cancer are called carcinogens and the process of cancer development is called carcinogenesis. Up to now, scientists have identified about two dozen chemicals or occupational exposures which appear to be definitely carcinogenic to humans. Some of the most familiar are tobacco smoke and asbestos. In addition, there are a number of chemicals that cause cancer in animals and are suspected of being human carcinogens. Since not all chemicals have been tested at present, it is possible that the number of known and suspect human carcinogens will increase in the future.

It must be remembered, however, that as with all toxic effects, the dose or amount of exposure is critical. Just as a small enough amount of cyanide will not lead to death, smoking one cigarette will not lead to lung cancer. Thus, in order to decide on the risk that a particular carcinogen poses, it is important to determine how much of the chemical will cause how many cases of cancer in a specified population. This value can then be compared to what is considered an acceptable risk. Currently, the generally accepted increase in risk of cancer is one additional cancer in one million people. A few exceptions to this criterion are made in the cases of food additives, including pesticides that are considered as food additives, where no amount of carcinogen is allowed (the Delaney Clause, as documented in the Federal Food, Drug and Cosmetic Act) and drinking water where a goal of zero contamination for carcinogens has been set.

CARCINOGEN TESTING

Once an acceptable risk for a carcinogen has been established (usually by the Environmental Protection Agency for environmental contaminants), there remains the problem of determining what dose or amount of chemical will lead to this risk. There are two types of studies that are used to make this determination: (1) investigations of human populations (epidemiology) and (2) experiments on laboratory animals. Each of these types of studies has its advantages and disadvantages and both have some degree of uncertainty, no matter how much evidence is gathered.

EPIDEMIOLOGICAL STUDIES IN HUMAN POPULATIONS

Investigations of human populations, in an attempt to establish the relationship between environmental factors and health, are called epidemiological studies. Scientists examine selected populations to single out particular exposures that might be related to toxic effects; in this case, cancer. Occupational groups, such as factory workers in a particular industry, are often studied for two reasons. One is that their exposures to the toxic compounds are generally higher than other people's so that a higher incidence of cancer is expected, if it occurs. A higher incidence is obviously easier to detect. The second reason is that their exposure to a specific chemical is often unique and can more easily be distinguished from exposures to many other chemicals that are used in daily life.

Through epidemiological studies among industrial worker populations, it was possible to show that asbestos is linked to lung cancer, vinyl chloride to a rare form of liver cancer, and benzene to leukemia. There have also been suggestions that pesticide exposure to farmers might lead to cancer but the results are not clear cut and there is still much controversy about the epidemiological studies which have been performed on these populations. Even in well-documented cases, it is not possible to use epidemiology to establish the exact risk of exposure to specific levels (concentrations) of these chemicals.

LABORATORY ANIMAL STUDIES

Laboratory studies have several advantages over epidemiological studies. Studies on laboratory animals are often easier to interpret because chemicals can be studied one at a time; very high doses can be administered; other chemicals and environmental factors can be eliminated or controlled; and animals can be sacrificed during the course of the study. The disadvantages are that it is not known how to apply these high dose results to much lower dose exposures that happen in the real world. Equally perplexing is how animal results can be applied to human populations. In extrapolating from high to low doses and from animals to humans, regulatory agencies have taken the approach of trying to be as conservative as possible, i.e., of trying to leave a large margin of safety so that even if

the studies are in error, human health (usually of the most sensitive groups in the population such as young children and pregnant women) will be protected.

As a result, the acceptable exposure levels (published in the Federal Register for each carcinogen) usually represent what is called the "worst case" exposure. An assumption made in the calculation of worst case exposure levels is that humans will be exposed to the same concentration of the chemical every day of their lives for seventy years. As a result, the published acceptable risk level does not necessarily represent the "safe level" but rather a target level with the expectation that the true risk to exposure is less than the published value. Remember that the exposure criteria are guidelines for the protection of sensitive elements of the population and are calculated with many factors of uncertainty (the relationship of animal toxicity to human toxicity for instance).

SUMMARY

Epidemiological investigations are used to establish links between a particular chemical and cancer in only a few cases and cannot be employed to determine the exact levels at which cancer will occur. On the other hand, laboratory animal studies provide a way of detecting the carcinogenicity of a large number of chemicals and can provide numerical values for cancer risks. However, the relevance of the animal high-dose results to low doses or to humans is not clear.

In light of these considerations, it is not possible to determine the exact cancer risk for any human population, much less any individual. Public policy makers have tried to use worst-case analyses to be as protective of human health as possible. To minimize cancer, regulations have been designed to reduce population exposure to known human carcinogens as much as possible. In the case of known animal carcinogens, minimizing exposure is also a regulatory goal. However, since many of these chemicals are also quite beneficial to society, there are questions as to how much exposure reduction can be achieved without eliminating the benefits of these chemicals. Achieving a balance of risk and benefit is especially difficult when the uncertainties involved in determining the actual risk to humans is considered.

At present, there are a number of pesticides known to be animal carcinogens. None have been shown absolutely to be human carcinogens. Exposure to pesticides that are probable human carcinogens can be minimized through proper protective equipment and proper storage, use and disposal of these pesticides. These measures not only protect the pesticide applicator but also the general public, which consumes foods treated with pesticides or spends time in buildings treated with these chemicals.

Chapter 7 - ECOLOGICAL EFFECTS

INTRODUCTION

Chemicals released into the environment may have a variety of adverse ecological effects. Ranging from fish and wildlife kills to forest decline, ecological effects can be long-term or short-lived changes in the normal functioning of an ecosystem, resulting in economic, social, and aesthetic losses. These potential effects are an important reason for regulation of pesticides, toxic substances, and other sources of pollution.

WHAT IS AN ECOSYSTEM?

The physical environment along with the organisms (biota) inhabiting that space, make up an ecosystem. Some typical examples of ecosystems include: a farm pond, a mountain meadow, and a rain forest.

An ecosystem follows a certain sequence of processes and events through the days, seasons, and years. The processes include the birth, growth, reproduction, and death of biota in that particular ecosystem, but also the interactions between species and physical characteristics of the geological environment. From these processes the ecosystem gains a recognizable structure and function, and matter and energy are cycled and flow through the system. Over time, better adapted species come to dominate; entirely new species may change, perhaps in a new or altered ecosystem.

THE ORGANIZATION OF ECOSYSTEMS

The basic level of ecological organization is with the individual, a single plant, insect or bird. The definition of ecology is based on the interactions of organisms with their environment. In the case of an individual, it would entail the relationships between that individual and numerous physical (rain, sun, wind, temperature, nutrients, etc.) and biological (other plants, insects, diseases, animals, etc.) factors. The next level of organization is the population. Populations are no more than a collection of individuals of the same species within an area or region. We can see populations of humans, birch trees, or sunfish in a pond. Population ecology is concerned with the interaction of the individuals with each other and with their environment.

The next, more complex, level of organization is the community. Communities are made up of different populations of interacting plants, animals, and microorganisms also within some defined geographic area. Different populations within a community interact more among themselves than with populations of the same species in other communities, therefore, there are often genetic differences between members of two different communities. The populations in a community have evolved together, so that members of that community provide resources (nutrition, shelter) for each other.

The next level of organization is the ecosystem. An ecosystem consists of different communities of organisms associated within a physically defined space. For example, a forest ecosystem consists of animal and plant communities in the soil, forest floor, and forest canopy, along the stream bank and bottom, and in the stream. A stream bottom community, for example, will have various fungi and bacteria living on dead leaves and animal wastes, protozoans and microscopic invertebrates feeding on these microbes, and larger invertebrates (worms, crayfish) and vertebrates (turtles, catfish). Each community functions somewhat separately, but are also linked to the others by the forest, rainfall, and other interactions. For example, the stream community is heavily dependent upon leaves produced in the surrounding trees falling into the stream, feeding the microbes and other invertebrates. For another example, the rainfall and groundwater flow in a surrounding forest community greatly affects the amount and quality of water entering the stream or lake system.

Terrestrial ecosystems can be grouped into units of similar nature, termed biomes (such as a "deciduous forest," "grassland," "coniferous forest," etc.), or into a geographic unit, termed landscapes, containing several different types of ecosystems. Aquatic ecosystems are commonly categorized on the basis of whether the water is moving (streams, river basins) or still (ponds, lakes, large lakes) and whether the water is fresh, salty (oceans), or brackish (estuaries). Landscapes and biomes (and large lakes, river basins, and oceans) are subject to global threats of pollution (acid deposition, stratospheric ozone depletion, air pollution, the greenhouse effect) and human activities (soil erosion, deforestation).

ADVERSE EFFECTS ON ECOSYSTEMS

While many natural forces -- drought, fire, flood, frost or species migration -- can affect it, an ecosystem will usually continue to function in a recognizable way. For instance, a pond ecosystem may go through flood or drought but continues to be a pond. This natural resilience of ecosystems enables them to resist change and recover quickly from disruption.

On the other hand, toxic pollutants and other non-natural phenomena can overwhelm the natural stability of an ecosystem and result in irreversible changes and serious losses, as illustrated by the following examples:

- decline of forests, due to air pollution and acid deposition;
- loss of fish production in a stream, due to death of invertebrates from copper pollution;
- loss of timber growth, due to nutrient losses caused by mercury poisoning of microbes and soil insects;
- decline and shift in age of eagle and hawk (and other top predator) populations, due to the effects of DDT in their food supply on egg survival;
- losses of numbers of species (diversity) in ship channels subjected to repeated oil spills;
- loss of commercially valuable salmon and endangered species (bald eagle, osprey) from forest applications of DDT.

Each of these pollutant-caused losses has altered ecosystem processes and components and thus affected aesthetic and commercial value of an ecosystem.

Usually, adverse ecological effects take place over long period of time or even at some distance from the point of release of a chemical. For example, DDT, though banned for use in the United States for over twenty years, is still entering the Great Lakes ecosystem through rainfall and dust from sources half way around the world. The long-term effects and overall impacts of new and existing chemicals on ecosystems can only be partially evaluated by current laboratory testing procedures. Nevertheless, through field studies and careful monitoring of chemical use and biological outcome, it is possible to evaluate the short-term and long-term effects of pesticides and other chemicals.

ADVERSE ECOLOGICAL EFFECTS ON COMMUNITIES

Scientists are most concerned about the effects of chemicals and other pollutants on communities. Short-term and temporary effects are much more easily measured than long-term effects of pollutants on ecosystem communities. Understanding the impact of effects requires knowledge of the time course and variability of these short-term changes.

Pollutants may adversely affect communities by disrupting their normal structure and delicate interdependencies. The structure of a community includes its physical system, usually created by the plant life and geological processes, as well as the relationships between its populations of biota.

For example, a pollutant may eliminate a species essential to the functioning of the entire community; it may promote the dominance of undesirable species (weeds, trash fish); or it may simply decrease the numbers and variety of species present in the community. It may also disrupt the dynamics of the food webs in the community by breaking existing dietary linkages between species. Most of these adverse effects in communities can be measured through changes in productivity in the ecosystem. Under natural stresses (for example, unusual temperature and moisture conditions), the community may be unable to tolerate effects of a chemical otherwise causing no harm.

An important facet of biological communities is the number and intensity of interactions between species. These interactions make the community greater than simply the sum of its parts. The community is stronger than its populations, and the ecosystem is more stable than its communities. A seriously altered interaction may adversely affect all the species dependent on it. Even so, some ecosystem properties or functions (such as nutrient dynamics) can be altered by chemicals without apparent effects on populations or communities. Thus, an important part of research in ecological effects is concerned with the relative sensitivity of ecosystems, communities, and populations to chemicals and to physical stresses.

Consider the effects of spraying an orchard with an insecticide when bees and other beneficial insects may be present and vulnerable to the toxicant. This practice is both economically and ecologically unsound, since it would deprive all plants in the area of pollinators and disrupt control of plant pests by their natural enemies. Advanced agricultural practices, such as integrated pest management (IPM), avoid these adverse effects through appropriate timing and selection of sprays in conjunction with non-chemical approaches to insect control.

Effects of chemicals on communities can be measured in laboratory model ecosystem (microcosm) studies, in intermediate sized systems (mesocosms, engineered field systems, open-top plant chambers, field pens), and in full

field trials. Thus, data gathered about effects of chemicals on processes and species can be evaluated in various complex situations that reflect the real world.

ADVERSE EFFECTS ON SPECIES

Most information on ecological effects has been obtained from studies on single species of biota. These tests have been performed in laboratories under controlled conditions and chemical exposures, usually with organisms reared in the laboratory representing inhabitants of natural systems. Most tests are short-term, single exposures (acute toxicity assays), but long-term (chronic) exposures are used as well. Although such tests reveal which chemicals are relatively more toxic, and which species are relatively more vulnerable to their effects, these tests do not disclose much about either the important interactions noted above or the role of the range of natural conditions faced by organisms in the environment.

Generally, the effects observed in these toxicity tests include reduced rates of survival or increased death rates; reduced growth and altered development; reduced reproductive capabilities, including birth defects; changes in body systems, including behavior; and genetic changes. Any of these effects can influence the ability of species to adapt and respond to other environmental stresses and community interactions.

Environmental toxicology studies performed on species in the laboratory provide the basis for much of the current regulation of pollutants and have allowed major improvements in environmental quality. However, these tests yield only a few clues to effects on more complex systems. Long-term studies and monitoring of ecological effects of new and existing chemicals released into the environment are needed in order to create understanding of potential adverse ecological effects and their consequences.

SUMMARY

Adverse ecological effects from environmental pollutants occur at all levels of biological organization, but most information about these effects has been obtained with single species. The effects can be global or local, temporary or permanent, or short-lived (acute) or long-term (chronic). The most serious effects involve loss in production, changes in growth, development and/or behavior, altered diversity or community structure, changes in system processes (such as nutrient cycling), and losses of valuable species. These ecological losses in turn may be economically, aesthetically, or socially important. Hence, ecological effects are of serious concern in regulating pollutants and a variety of tests have been devised to help evaluate the potential for adverse ecological effects. Developing an understanding of how these tests and other information can be used to prevent environmental problems caused by pollutants is the basis for ecological risk assessment research.