Raja Narendralal Khan Women's College (Autonomous)

HUMAN PHYSIOLOGY

ENVIRONMENTAL TOXICOLOGY

PHY-403,UNIT-37,MODULE-IV

4TH SEM (PG) STUDY MATERIAL
III

By-

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ENVIRONMENTAL TOXICOLOGY

Toxic responses to foreign compounds:

There are many ways in which an organism may respond to a toxic compound, and the type of response depends upon numerous factors. Although many of the toxic effects of foreign compounds have a biochemical basis, the expression of the effects may be very different. Thus, the development of tumours may be one result of an attack on nucleic acids, another might be the birth of an abnormal offspring. The interaction of a toxic compound with normal metabolic processes may cause a physiological response such as muscle paralysis, or a fall in blood pressure, or it may cause a tissue lesion in one organ. The covalent interaction between a toxic foreign compound and a normal body protein may in some circumstances cause an immunological response, in others a tissue lesion.

Thus, although all these toxic responses may have a biochemical basis, they have been categorized according to the manifestation of the toxic effect. Therefore although there will be overlap between some of the types of toxic response, for the purposes of this discussion it is convenient to divide them into the following:

1. direct toxic action: tissue lesions
2. pharmacological, physiological and biochemical effects
3. teratogenesis
4. immunotoxicity
5. mutagenesis
6. carcinogenesis.

Toxic responses may be detected in a variety of ways in animals and some of these have already been alluded to in previous chapters. Toxic responses may be the **all-or-none type** such as the death of the organism or they may be **graded responses.** Thus the main means of detection are:

A) Death: the LD_{50} assay has been utilized as an indicator of toxicity although it will be increasingly superseded by other assays;

B) Pathological change: this could be the development of a tumour or destruction. of tissue but it would be detectable by observation either macroscopically or microscopically;

C) Biochemical change: this might involve an effect on an enzyme such as inhibition or alteration in a particular metabolic pathway. Alternatively the appearance of an enzyme or other substance in body fluids may indicate leakage from tissue due to damage and be indicative of pathological change;

D) Physiological change: this could be measured in the whole conscious animal as for example a change in blood pressure, in temperature or in a response to a particular stimulus;

E) Changes in normal status: there are a number of *markers of toxicity* which are simple to determine yet indicate a toxic response. Thus changes in body weight, food and water intake, urine output and organ weight may all be sensitive indicators of either general

or specific toxicity. Thus animals often consume less food and lose weight after exposure to toxic compounds and increased organ weight may be due to a tumour, fluid or triglyceride accumulation, hypertrophy or enzyme induction. These changes may of course be confirmed by chemical, biochemical or histopathological measurements.

Direct toxic action: tissue lesions

Some toxic compounds cause *direct* damage to tissues leading to the death of some or all of the cells in an organ for instance. The damage may be *reversible* or *irreversible* and the overall toxic response will depend on many factors including the *importance* of the particular tissue to the animal, the degree of *specialization* and its *reserve* functional capacity and ability to *repair* the damage.

TARGET ORGAN TOXICITY

Although any organ or tissue may be a target for a toxic compound, such compounds often damage specific organs. Therefore it is instructive first to examine the principles underlying the susceptibility of certain organs to damage by toxic substances.

Thus, there are a number of different reasons why an organ might be a target:

A) its blood supply

B) the presence of a particular enzyme or biochemical pathway the function or position of the organ

D) the vulnerability to disruption or degree of specialization

E) the ability to repair damage

F) the presence of particular uptake systems

G) the ability to metabolize the compound and the balance of toxication/ detoxication systems

H) binding to particular macromolecules.

Factors affecting toxic responses: metabolism

The **biotransformation** of foreign compounds, however, attempts to convert such lipophilic substances into more polar, and consequently more readily excreted metabolites. The exposure of the body to the compound is hence reduced and potential toxicity decreased. This process of biotransformation is therefore a crucial aspect of the disposition of a toxic compound *in vivo.* The metabolic fate of a compound can therefore have an important bearing on its toxic potential, disposition in the body and eventual excretion.

The primary results of biotransformation are therefore:

1. the parent molecule is transformed into a more polar metabolite, often by the addition of ionisable groups
2. molecular weight and size are often increased

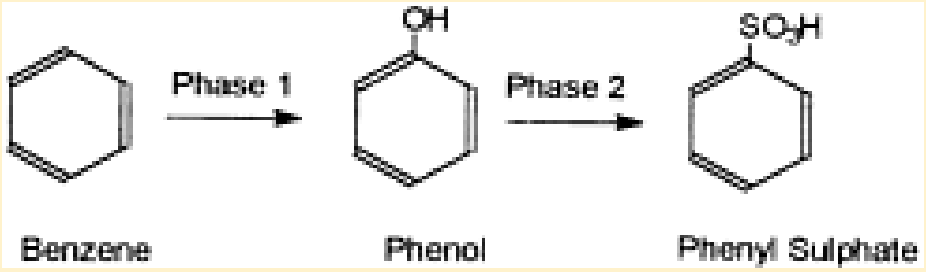
3. the excretion is facilitated, and hence elimination of the compound from the tissues and the body is increased.

The consequences of metabolism are:

a. the biological half-life is decreased
b. the duration of exposure is reduced
c. accumulation of the compound in the body is avoided
d. the biological activity may be changed
e. the duration of the biological activity may be affected.

Types of metabolic change

Metabolism can be simply and conveniently divided into two phases: **phase 1** and **phase 2**. Phase 1 is the alteration of the original foreign molecule so as to add on a functional group which can then be conjugated in phase 2. This can best be understood by this figure (metabolism of benzene):



The foreign molecule is **benzene,** a highly lipophilic molecule which is not readily excreted from the animal except in the expired air as it is volatile. Phase 1 metabolism converts benzene into a variety of metabolites, but the major one is phenol. The insertion of a hydroxyl group allows a phase 2 conjugation reaction to take place with the polar sulphate group being added. Phenyl sulphate, the final metabolite is very water soluble and is readily excreted in the urine. Most biotransformations can be divided into phase 1 and phase 2 reactions, although the products of phase 2 biotransformations may be further metabolized in what is sometimes termed **phase 3** reactions.

If the foreign molecule already possesses a functional group suitable for a phase 2 reaction, a phase 1 reaction will be unnecessary. Thus, if phenol is administered to an animal then it may immediately undergo a phase 2 reaction, such as conjugation with sulphate. Alternatively it may undergo another
phase 1 type of reaction. The major types of reaction are shown in following table:

Phase 1	Phase 2	Phase 3
Oxidation	Sulphation	Further metabolism
Reduction	Glucuronidation	of glutathione
Hydrolysis	Glutathione conjugation	conjugates
Hydration	Acetylation	
Dehalogenation	Amino acid conjugation	
	Methylation	

Generally, therefore, the function of phase 1 reactions is to *modify* the *structure* of a xenobiotic so as to introduce a *functional group* suitable for conjugation with glucuronic acid, sulphate or some other highly-polar moiety, so making the entire molecule water-soluble.

Phase 1 reactions

The major phase 1 reactions are oxidation, reduction and hydrolysis.

1. **OXIDATION**

For foreign compounds the majority of oxidation reactions are catalysed by **mono oxygenase enzymes** found in the SER and known as **microsomal enzymes**. Other enzymes involved in the oxidation of xenobiotics are found in other organelles such as the mitochondria and the cytosol. Thus amine oxidases located in the mitochondria, xanthine oxidase, alcohol dehydrogenase in the cytosol, the prostaglandin synthetase system and various other peroxidases may all be involved in the oxidation of foreign compounds.

Microsomal oxidations may be subdivided into: aromatic hydroxylation; aliphatic hydroxylation; alicyclic hydroxylation; heterocyclic hydroxylation; *N*-, S- and *O*-dealkylation; *N*-oxidation; *N*hydroxylation; S-oxidation; desulphuration; and deamination; and dehalogenation. Non-microsomal oxidations may be subdivided into: amine oxidation; alcohol and aldehyde oxidation; dehalogenation; purine oxidation; and aromatization.

Microsomal oxidations:

CYTOCHROMES P-450 MONO-OXYGENASE SYSTEM

The majority of these reactions are catalysed by one enzyme system, the cytochromes P-450 monooxygenase system, which is located particularly in the SER of the cell. The enzyme system is isolated in the so-called **microsomal** fraction which is formed from the endoplasmic reticulum when the cell is homogenized and fractionated by differential ultracentrifugation. Microsomal vesicles are thus fragments of the endoplasmic reticulum. in which most of the enzyme activity is retained. The endoplasmic reticulum is composed of a
convoluted network of channels and so has a large surface area. Apart from cytochromes P-450, the endoplasmic reticulum has many enzymes and functions besides the metabolism of foreign compounds.

These include the synthesis of proteins and triglycerides, and other aspects of lipid metabolism and fatty acid metabolism. Specific enzymes present on the endoplasmic reticulum include cholesterol esterase, azo reductase, glucuronosyl transferase, NADPH cytochromes P-450 reductase and NADH cytochrome
b5 reductase and cytochrome b5. A FAD-containing mono-oxygenase is also found in the endoplasmic reticulum and this is discussed later in this chapter.

The cytochromes P-450 mono-oxygenase system is actually a collection of *isoenzymes* all of which possess an iron protoporphyrin IX as the prosthetic group. The monomer of the enzyme has a molecular weight of 45 000–55 000. The enzyme is membrane bound within the endoplasmic reticulum.

Cytochromes P-450 is closely associated with another vital component of the system, **NADPH cytochrome P-450 reductase**. This is a **flavoprotein** which has 1 mol of FAD and 1 mol of FMN per mol of apoprotein. The monomeric molecular weight of the enzyme is 78 000. The enzyme transfers two electrons to cytochromes P-450, but one at a time. There only seems to

be one reductase which serves a group of isoenzymes of cytochromes P-450, and consequently its concentration is 1/10 to 1/30 that of cytochromes P-450.

Phospholipid is also required in the enzyme complex, seemingly this is important for the integrity of the overall complex and the interrelationship between the cytochromes P-450 and the reductase. The individual components can be separated and reconstitution of these components results in a functional enzyme. The overall reaction is:

$$SH + O_2 + NADPH + H^+ \rightarrow SOH + H_2O + NADP^+$$

where S is the substrate. The reaction therefore also requires NADPH and molecular oxygen. The mechanism of action of cytochrome P-450 involves oxygen activation followed by abstraction of a hydrogen atom or an electron from the substrate and oxygen rebound (radical recombination).

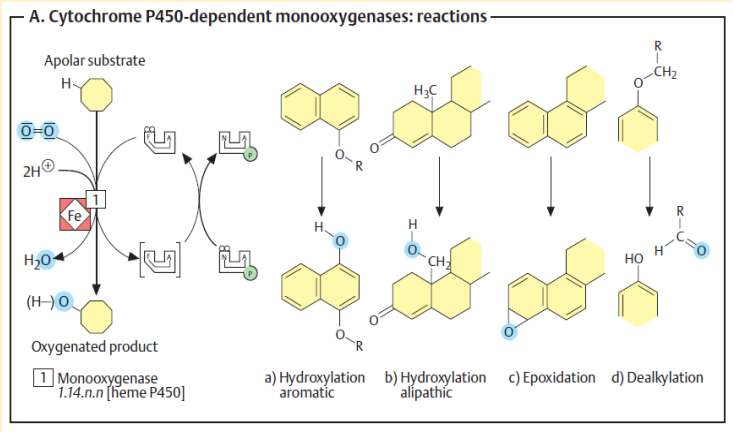
The sequence of metabolic reactions involve at least four distinct steps:

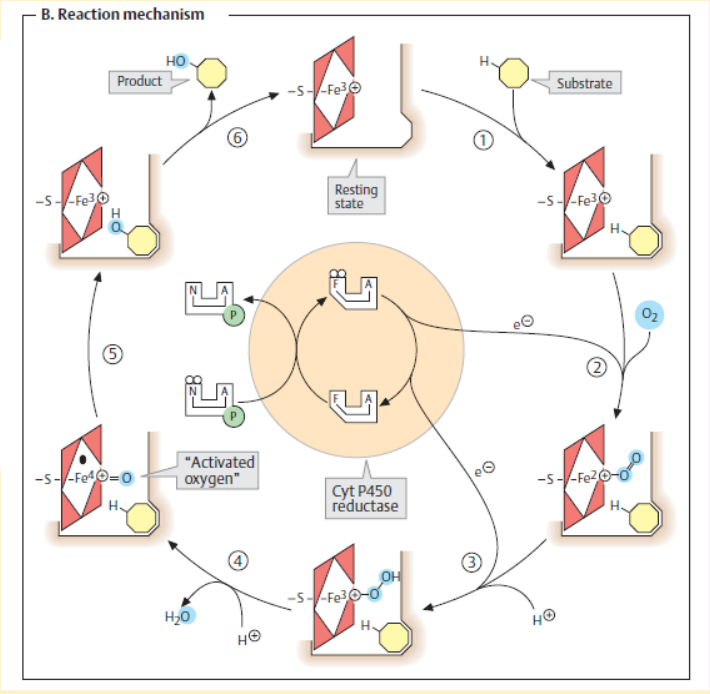
i addition of substrate to the enzyme

ii donation of an electron

iii addition of oxygen and rearrangement

iv donation of a second electron and loss of water.





Environmental Behaviour of Chemicals

When testing the toxicity of chemicals to organisms in the laboratory, the chemical is usually fed to or applied directly to the animal or incorporated into the medium in which the organism is living (i.e., soil, water, sediment). However, when a chemical is in the environment, it partitions among different media according to specific physical properties and chemical

behaviours. This directly influences the amount and form of the chemical to which organisms are exposed. Therefore, to conduct toxicity tests with the relevant chemical form and concentrations, the environmental transport and fate of chemicals must be understood.

Testing of chemicals for their toxic effects traditionally focused on safety and effects in humans using surrogate species. Beginning in the 1960s, the recognition th at chemicals in the environment can have effects on nonhuman receptors has led to the emergence of the subdiscipline of ecotoxicology. Subsequently, testing protocols with a wide range of surrogate species have been established to address questions concerning thresholds of toxic effects and mode of action. By expanding the number of species tested in assessing the toxicology of a chemical, we are able to predict and diagnose possible adverse environmental effects and also gain considerable insight into a toxicant's mechanism of action, organ-specific toxicity, and acute and long-term effects. Field protocols emphasize methods and indicators for determining the consequences of chemical exposure on species and their populations. In this chapter, we provide principles and examples of laboratory and field protocols that have been developed to understand the effects of chemicals in the environment.

Water Solubility and Lipophilicity

The most significant determinant of the transport and fate of a chemical in the environment is its water solubility. Highly soluble chemicals are transported through the hydrologic cycle and thus can be distributed over great distances from their points of introduction into the environment. Conversely, hydrophobic compounds tend to be more static and move little through the hydrologic cycle. Generally, the more water soluble the chemical, the less lipophilic it is, the less it is sorbed to soils and sediments, and the less it accumulates into biota. Water solubility is defined as the maximum amount of a chemical that can be freely dissolved in a given quantity of pure water at a particular temperature. It is important to note that even chemicals described in tables of physical constants as very insoluble may have sufficient water solubility to significantly influence their behaviour in the environment. It is particularly important to note that metals and other inorganics with low water solubility may be converted to more water-soluble forms in the environment or introduced as soluble salts. A derivative property of the water solubility and lipophilicity of a chemical is its octanol/water partition coefficient (K_{OW} or P), frequently reported as log K_{OW}. K_{OW} is defined as the ratio of the concentrations of a chemical in the water phase and in the *n*-octanol phase after equilibrating between equal volumes of the two solvents. It is a key property of a chemical for environmental considerations, as it is a predictor of soil and sediment adsorption and subsequent bioaccumulation in organisms and potential biomagnification through trophic transfer up the food chain. In designing studies to assess the toxicity of a chemical in model ecosystems, such as microcosms or mesocosms, the octanol/ water partition coefficient must be known or experimentally determined, so the behaviour of the chemical in the system can be predicted and the system designed appropriately.

Soil Adsorption

The extent of partitioning of a chemical between the solid and solution phases of a water-saturated soil or sediment is described by the soil sorption coefficient (*K* or *K*d) . It is determined experimentally using the Freundlich equation:

$$x/m = KC^{1/n}$$

where

x/m is the μg of chemical adsorbed per g of soil

C is the μg of chemical per mL of solution

K and *n* are constants for a particular soil type

The value of *n* must be determined experimentally but is frequently assumed to be 1. *K*OC, the soil sorption constant, is determined from *K* by dividing by the percent organic carbon in the soil and multiplying the result by 100. This constant is observed to be relatively independent of the type of soil or sediment and is the value most frequently used to describe the adsorption of a chemical to soil or sediment. Consideration of the soil adsorption of a chemical is extremely important for the proper design of ecological test systems.

Atmospheric Particulate Matter

When pollutant-contaminated soils become airborne, chemicals sorbed to the resulting particulate matter must be considered in some testing protocols. Such sorbed chemicals may be bioavailable in biological systems. Soil-derived particulate matter is composed of a mineral fraction and an organic matter fraction. Affinity of organic chemicals for the mineral fraction is believed to be low, but hydrophobic organic chemicals may have a high affinity for the organic portion. Once particulate matter is suspended in the atmosphere, gas/particle partitioning of adsorbed chemicals is a function of the vapor pressure of the chemical. Chemicals with a vapor pressure <10−6 atm will be primarily in the particle phase, while those with a vapor pressure >1 atm will partition primarily to the gas phase. Thus, under some testing conditions, the air quality of the test system must be controlled.

Vaporization

The vaporization of a chemical from a solid surface or a solution is an important mass-transfer process. Factors that control volatilization are diffusivity of the chemical, its water solubility, vapor pressure, the Henry's law constant, and temperature. Diffusivity is the rate of diffusion of a chemical through a medium and depends on the nature of the chemical itself and the nature of the medium through which it moves. Vapor pressure is the tendency of a liquid to change from the liquid to the gaseous state and is highly dependent on temperature.

The air/water interface is most important in environmental analyses, so the question of the ability of a chemical to diffuse across that interface is significant when evaluating its environmental behaviour. The tendency of a chemical to escape from solution is described by the Henry's law constant. Henry's law states that the solubility of a gas in a liquid is directly proportional to the pressure of the gas above the liquid at equilibrium: $H = P/C$, where C is measured in mol/m^3 and P is in atm; that is, H is the ratio of the saturation vapor pressure and the water solubility of the chemical. Units of H are most often reported as atm-m3/mol; however, if C is expressed in mol/L and P is expressed in mol/m^3, H is dimensionless. Under

these circumstances, the Henry's law constant is sometimes referred to as the air/water partition coefficient. Movement of chemicals in the environment occurs in the vapor state, as well as in solution or when adsorbed to particulate matter. The most important consideration in evaluating the extent of such movement is the Henry's law constant, because most chemicals will eventually be found in water solution, and their tendency to move into the air will have an impact on their potential for toxic effects in organisms that may be exposed. In the design of toxicology test methods, such considerations must be taken into account.

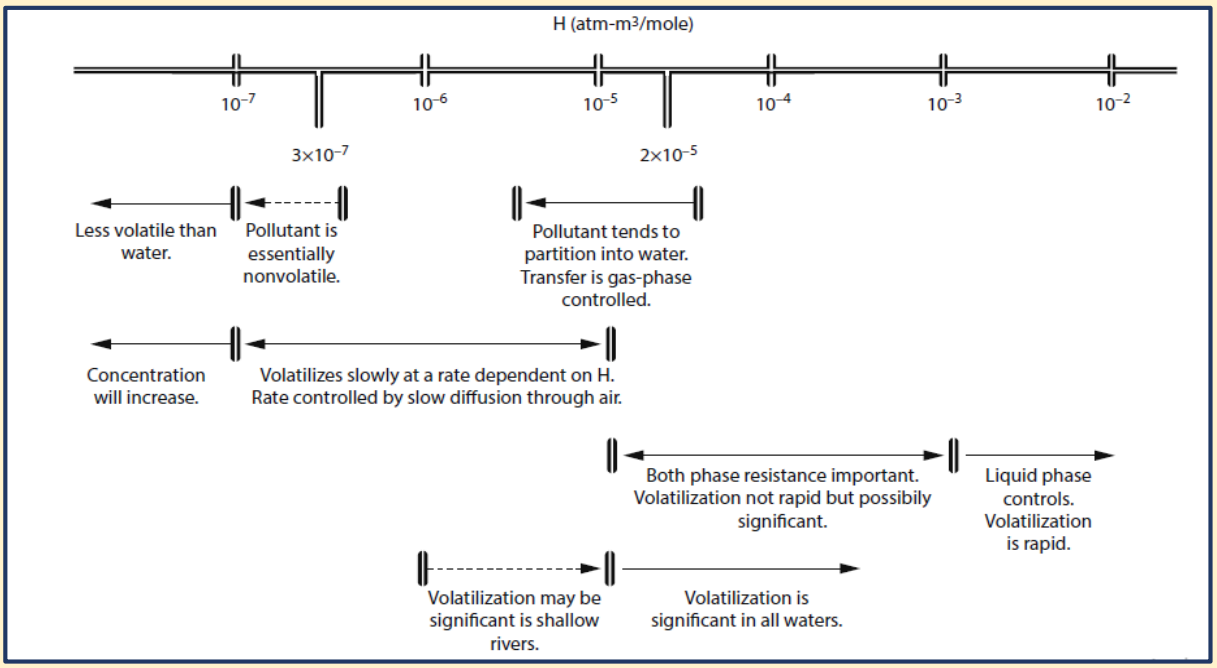


Fig: Volatility characteristics associated with various ranges of Henry's law constant. [From Smith, J.H. et al., *Environ. Sci. Technol.*, 14, 1332, 1980; Reproduced in Lyman, W.J. et al., *Handbook of Chemical Property Estimation Methods*, McGraw-Hill, New York, 1982.]

Bioaccumulation

Certain chemicals accumulate in organisms that are exposed in their environments. Some chemicals biomagnify in the food chain, such that higher trophic-level organisms have higher concentrations of the substance in their tissues than do their prey. There are now many well-known cases of chemicals biomagnifying in food webs to the point that toxic effects are exhibited in organisms that may have had no direct exposure to the chemical itself at its point of application; polychlorinated biphenols (PCBs), organochlorine insecticides, and mercury are a few examples. The tendency of a chemical to be more concentrated in an organism than the concentration in its environment is described by its bioaccumulation factor (BAF) or bioconcentration factor (BCF). The BAF or BCF is calculated by dividing the concentration of the chemical in the organism by the concentration of the chemical in the soil, sediment, or water in which it lives. BAFs assume that exposure has occurred through all potential routes, including direct uptake from the environmental medium and ingestion of contaminated foods. BCFs refer to water exposures and are applied typically only to aquatic organisms; however,

BCFs have been used considering pore-water transfer of chemicals to invertebrates in mesic soil environments. BCFs have units of water volume per unit tissue weight (e.g., mL/g) and can be viewed conceptually as the volume of water containing the amount of chemical in 1 g of tissue.

Biodegradation

As organic chemicals move in the environment, they are subjected to breakdown, primarily by microorganisms, in a process known as *biodegradation*. This process represents a significant loss mechanism in soil and sediments that can ultimately lead to mineralization of the compound—that is, degradation to carbon dioxide, water, and the inorganic forms of other elements the chemical may contain. Microorganisms are the primary converters of complex organic chemicals in to inorganic substances, although soil and sediment invertebrates also play a major role in the biodegradation process. In many instances, higher organisms are able to metabolize compounds, but they generally play a less significant role in environmental systems. Photochemical degradation and hydrolysis are other important abiotic degradation factors in the environment. Almost all degradative reactions in the environment are oxidative, reductive, hydrolytic, or conjugative. Biodegradation can take place in virtually any environmental situation, aerobic or anaerobic; thus, in a test system designed to assess the toxicity of a chemical, the potential for biodegradation must always be considered regardless of the presence or absence of oxygen. When testing chemicals in anything more complex than a single organism (i.e., the microcosms and mesocosms, discussed later in this chapter), the medium, whether water, sediment, soil, or a combination of these, plays an important role in the behaviour of the system. Organic matter in the medium strongly influences its microbial density, and microorganisms may comprise as much as 80% of the biomass of soil. The microbial community in turn determines how stable a xenobiotic chemical will be in the system.

Organic compounds can be divided into four groups according to their biodegradability: (1) usable immediately by an exposed organism as a nutrient or energy source, (2) usable by microorganisms following an acclimation period, and (3) degraded slowly or not at all. In the fourth group, compounds are subject to co-metabolic degradation, wherein a compound that does not provide a nutrient or energy source for the degrading organism is broken down in conjunction with the degradation of other substances. Ideally, when evaluating the effect of xenobiotics on complex systems, one needs to consider all aspects of the biology and chemistry of all of the organisms and chemicals present. The best design of such test systems demands the most complete knowledge possible of all the potential interactions of chemicals and organisms in the system.