PART VII

ENVIRONMENTAL TOXICOLOGY

CHAPTER 25

Analytical Methods in Toxicology

ROSS B. LEIDY

25.1 INTRODUCTION

Some 200,000 chemicals are synthesized annually worldwide, and the toxicity of most of them is unknown. Few of these chemicals reach the stage of further development and use, but those that do usually find their way into the environment. Some are persistent and remain adsorbed to soil particles or soil organic matter, some find their way into water through soil movement or aerial deposition, others are metabolized by microorganisms into compounds of greater toxicity that move up the food chain. Over time, their accumulation in higher life forms could result in debilitating alterations in metabolism, leading to illness. It might be years before such illness could be attributed to specific compounds because of the difficulty involved in identifying and quantitating them. The concern over the role of persistent organochlorines in the food chain and their possible role as human xenoestrogens is an example. The identification and quantitation of chemicals in both the environment and in living beings relies on the development of analytical techniques and instruments.

Advances in analytical techniques continue to multiply in all fields of toxicology, and as mentioned, many of these focus on the environmental area. Whether looking for new techniques to sample water or for an automated instrument to determine quantities of sulfur-containing compounds in air, such devices are available. In many instances, developments in environmental analyses are adaptable to experimental work related to drug toxicity, or in forensic medicine, to determine the cause of poisoning.

Although new techniques and instruments continue to enter the commercial market, the basic analytical process has not changed: define the research goal(s), develop a sampling scheme to obtain representative samples, isolate the compound(s) of interest, remove potential interfering components, and quantitate and evaluate the data in relation to the initial hypothesis. Based on the data generated, many options are available. For example, was the sampling scheme complete? Would further refinement of the analytical procedure be required? Should other sample types be analyzed? Thus it is obvious that within these general categories particular methods vary considerably depending on the chemical characteristics of the toxicant (Table 25.1).

A Textbook of Modern Toxicology, Third Edition, edited by Ernest Hodgson
ISBN 0-471-26508-X

Table 25.1 Typical Protocols for Analysis of Toxicants

	Toxicant		
Step	Arsenic	TCDD	Chlorpyrifos
Sampling	Grind solid sample homogenize tissue to homogeneity; subsample	Grind solid sample or homogenize tissue to homogeneity; subsample	Grind solid sample or homogenize tissue to homogeneity; subsample Soxhlet extract with hexane:acetone (1:1)
Extraction and cleanup	Dry ash; redissolve residue; generate arsine and absorb into solution	Extract with ethanol and KOH; remove saponified lipids; column chromatography on H_2S0_4/silica gel followed by basic alumina and then by $AgN0_3$/silica gel followed by basic alumina; reverse-phase HPLC	Remove co-extractives on Florisil using ether: petroleum ether
Analysis	AA spectroscopy	GC/MS	GC/NPD or FPD

Source: Modified from R. J. Everson and F. W. Oehme, *Analytical Toxicology Manual*, New York: KS American College of Veterinary Toxicologists, 1981.
Note: TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; GLC or GC, gas-liquid chromatography; AA, atomic adsorption; NPD, nitrogen phosphorus detector; FPD, flame photometric detector; GC/MS, gas chromatography/mass spectrometry.

This chapter is concerned with the sampling, isolation, separation, and measurement of toxicants, including bioassay methods. Bioassay does not measure toxic effects; rather, it is the quantitation of the relative effect of a substance on a test organism as compared with the effect of a standard preparation of a basic toxicant. Although bioassay has many drawbacks, particularly lack of specificity, it can provide a rapid analysis of the relative potency of toxicants in environmental samples.

25.2 CHEMICAL AND PHYSICAL METHODS

25.2.1 Sampling

Even with the most sophisticated analytical equipment available, the resulting data are only as representative as the samples from which the results are derived. This is particularly true for environmental samples. In sampling, care must be taken to ensure that the result meets the objectives of the study. Often special attention to sampling procedures is necessary. Sampling accomplishes a number of objectives, depending on the type of area being studied. In environmental areas (e.g., wilderness regions, lakes, rivers) sampling can provide data not only on the concentration of pollutants but also on the extent of contamination. In urban areas, sampling can provide information on the types of pollutants, to which one is exposed, by dermal contact, by inhalation, or by ingestion over a given period of time.

In industrial areas, hazardous conditions can be detected and sources of pollution can be identified. Sampling is used in the process of designing pollution controls and can provide a chronicle of the changes in operational conditions as controls are implemented. Another important application of sampling in industrial areas in the United States is the documentation of compliance with existing Occupational Safety and Health Administration (OSHA) and US Environmental Protection Agency (US EPA) regulations. The many methods available for sampling the environment can be divided into categories of air, soil, water, and tissue sampling. The fourth category is of particular interest in experimental and forensic studies.

Air. Most pollutants entering the atmosphere come from fuel combustion, industrial processes, and solid waste disposal. Additional miscellaneous sources, such as nuclear explosions, forest fires, dusts, volcanoes, natural gaseous emissions, agricultural burning, and pesticide drift, contribute to the level of atmospheric pollution. To affect terrestrial animals and plants, particulate pollutants must be in a size range that allows them to enter the body and remain there; that is, they must be in an aerosol (defined as an airborne suspension of liquid droplets) or on solid particles small enough to possess a low settling velocity. Suspensions can be classified as liquids including fogs (small particles) and mists (large particles) produced from atomization, condensation, or entrapment of liquids by gases; and solids including dusts, fumes, and smoke produced by crushing, metal vaporization, and combustion of organic materials, respectively.

At rest, an adult human inhales 6 to 8 L of air each minute (1 L = O.OO1 m^3) and, during an 8-hour workday, can inhale from 5 to 20 m^3 depending on the level of physical activity. The optimum size range for aerosol particles to get into the lungs and remain there is 0.5 to 5.0 μm. As instrumentation used to collect atmospheric dust have become more precise, particulate matter (PM) in the size range of 2.5 to 10 μm have come under increasing scrutiny, because many potential toxicants are adsorbed to their surfaces. These particles are inhaled and will remain in the lungs and allow the compounds to pass into the bloodstream.

Thus air samplers have been miniaturized and adsorbents have been developed to collect either particulate matter in the size range most detrimental to humans or to "trap" organic toxicants from air. An air sampler generally consists of an inlet to direct air through a filter to entrap particles that might be of interest (e.g., dust); through the adsorbent, which collects organic vapors, a flowmeter and valve to calibrate airflow, and a pump to pull air through the system. Personnel samplers are run by battery power and can be attached to an individual's clothing, thus allowing continual monitoring while performing assigned tasks in the work environment. This allows the estimation of individual exposure.

Many air samplers use various types of filters to collect solid particulate matter, such as asbestos, which is collected on glass fiber filters with pores 20 μm or less in diameter. Membrane filters with pores 0.01 to 10 μm in diameter are used to collect dusts and silica. Liquid-containing collectors, called impingers, are used to trap mineral dusts and pesticides. Mineral dusts are collected in large impingers that have flow rates of 10 to 50 L of air per minute, and insecticides can be collected in smaller "midget" impingers that handle flows of 2 to 4.5 L of air per minute. Depending on the pollutant being sought, the entrapping liquid might be distilled water, alcohol, ethylene glycol, hexylene glycol (2-methyl, 2,4-pentane diol) or some other solvent. Because of the ease of handling and the rapid desorption of compounds, polyurethane foam (PUF)

has become a popular trapping medium for pesticides and is rapidly replacing the use of midget impingers. A large volume air sampler has been developed by the US EPA for detection of pesticides and polychlorinated biphenyls (PCBs). Air flows at rates of around 225.0 L/min are drawn through a PUF pad, and the insecticides and PCBs are trapped in the foam. Small glass tubes approximately 7.0×0.5 cm in diameter containing activated charcoal are used to entrap organic vapors in air.

A number of specialty companies have and are continuing to develop adsorbents to collect organic molecules from air samples. Industrial chemicals resulting, from syntheses or used in production processes, pesticides and emissions from exhaust towers are monitored routinely with commercially available adsorbents. Personnel monitoring can be accomplished without a pump using a system composed of a porous membrane through which air diffuses and compounds of interest are collected by an adsorbent.

Minute quantities of gaseous pollutants (e.g., CO_2, HNO_3), are monitored with direct reading instruments, using infrared spectroscopy, and have been in use for a number of years. These instruments passively monitor large areas and rely on extensive statistical evaluations to remove substances like water vapor, which can mask the small quantities of these pollutants. Research into the millimeter/submillimeter area of spectroscopy coupled with Russian technologies is leading to the development of a direct reading instrument that will quantitate any atmospheric gas or a mixture of gases containing a dipole moment within 10 seconds, regardless of the presence or quantity of water vapor in the atmosphere. Such devices are expected to be commercially available within the next five years.

Soil. When environmental pollutants are deposited on land areas, their subsequent behavior is complicated by a series of simultaneous interactions with organic and inorganic components, existing liquid-gas phases, microscopic organisms, and other soil constituents. Depending on the chemical composition and physical structure, pollutants might remain in one location for varying periods of time, be absorbed into plant tissue, or move through the soil profile from random molecular motion. Movement is also affected by mass flow as a result of external forces such as the pollutant being dissolved in or suspended in water or adsorbed onto both inorganic and organic soil components. Thus sampling for pollutants in soils is complex and statistical approaches must be taken to ensure representative samples.

To obtain such samples, the chemical and physical characteristics of the site(s) must be considered, as well as possible reactions between the compound(s) of interest and soil components and the degree of variability (i.e., variation in soil profiles) within the sampling site. With these data, the site(s) can then be divided into homogeneous areas and the required number of samples can be collected. The required number of samples depends on the functions of variance and degree of accuracy. Once the correct procedure has been determined, sampling can proceed.

Many types of soil samplers are available, but coring devices are preferable because this collection method allows determination of a pollutant's vertical distribution. These devices can be either stainless steel tubes, varying in both diameter from 2.5 to 7.6 cm and length from 60 to 100 cm (hand operated). Large, mechanically operated boring tubes, 200 cm in length are also used. It is possible to sample to uniform depths with these devices, and one can subdivide the cores into specific depths (e.g., 0–7.6 cm, 7.6–15.2 cm, etc.) to determine movement. Another type of coring device is a wheel to which are attached tubes so that large numbers of small subsamples can be collected,

thus allowing a more uniform sampling over a given area. Soils from specific depths can be collected using a large diameter cylinder (ca. 25 cm) that incorporates a blade to slice a core of soil after placing the sampler at the desired depth.

Water. Many factors must be considered to obtain representative samples of water. The most important are the pollutant and the point at which it entered the aquatic environment. Pollutants can be contributed by agricultural, industrial, municipal, or other sources, such as spills from wrecks or train derailments. The prevailing wind direction and speed, the velocity of stream or river flow, temperature, thermal and salinity stratification, and sediment content are other important factors.

Two questions, where to monitor or sample and how to obtain representative samples are both important. Surface water samples often are collected by automatic sampling devices controlled by a variety of sensors. The simplest method of collecting water is the "grab" technique, whereby a container is lowered into the water, rinsed, filled, and capped. Specialized samplers frequently are used to obtain water at greater depths.

With the implementation in the United States of the Clean Water Act of 1977, continuous monitoring is required to obtain data for management decisions. A number of continuous monitoring wells are in operation throughout the United States. Sampling from potable wells can be accomplished by collecting from an existing tap, either in the home or from an outside fixture. However, multistep processes are required to collect samples from wells used to monitor pollutants. Standing water must be removed after measuring the water table elevation. If wells are used to monitor suspected pollutants, two criteria are used to determine the amount of water removed prior to sampling: conductivity and pH. Removal of a specific number of well volumes by bailers or pumps is done until both pH and conductivity are constant. A triple-rinsed bottle is then used to collect the sample.

Because large numbers of samples can be generated by such devices, collectors containing membranes with small pores (e.g., 45.0 μm) to entrap metal-containing pollutants, cartridges containing ion-exchange resins, or long-chain hydrocarbons (e.g., C_{18}) bonded to silica to adsorb organic pollutants. These devices often are used to diminish the number and bulk of the samples by allowing several liters of water to pass through and leave only the pollutants entrapped in a small cylinder or container. In addition disk technologies use a filter containing a Teflon matrix in which C_{18} hydrocarbon chains are embedded to concentrate pollutants as water is passed through the membrane. Polar solvents (e.g., methanol) are used to elute them from the disk.

Once samples have been collected, they should be frozen immediately in solid CO_2 (dry ice) and returned to the laboratory. If they are not analyzed at that time, they should be frozen at temperatures of $-20^{\circ}C$ or lower. Sufficient head space must be left in the container to prevent breakage.

Tissues. When environmental areas are suspected of being contaminated, surveys of plants and animals are conducted. Many of the surveys, conducted during hunting and fishing seasons by federal and state laboratories, determine the number of animals killed and often, organs and other tissues are removed for analysis of suspected contaminants. Sampling is conducted randomly throughout an area, and the analyses can help determine the concentration, extent of contamination within a given species and areas of contamination.

Many environmental pollutants are known to concentrate in bone, certain organs, or specific tissues (e.g., adipose). These organs are removed from recently killed animals

for analysis. In many instances, the organs are not pooled with others from the same species but are analyzed separately as single sub-samples to determine the extent of possible contamination in the area sampled.

When plant material is gathered for analysis, it is either divided into roots, stems, leaves, and flowers and/or fruit or the whole plant is analyzed as a single entity. Pooling of samples from a site can also provide a single sample for analysis. The choice depends on the characteristics of the suspected contaminant.

25.2.2 Experimental Studies

Experimental studies, particularly those involving the metabolism or mode of action of toxic compounds in animals (or, less often, plants), can be conducted either in vivo or in vitro. Because organisms or enzyme preparations are treated with known compounds, the question of random sampling techniques does not arise as it does with environmental samples. Enough replication is needed for statistical verification of significance, and it should always be borne in mind that repeated determinations carried out on aliquots of the same preparation do not represent replication of the experiment; at best, they test the reproducibility of the analytical method.

In environmental studies, the analyst is concerned with stable compounds or stable products; in metabolic studies, the question of reactive (therefore unstable) products and intermediates is of critical concern. Thus the reaction must be stopped, and the sample must be processed using techniques that minimize degradation. This is facilitated by the fact that the substrate is known, and the range of possible products can be determined by a variety of methods.

The initial sampling step is to stop the reaction, usually by a protein precipitant. Although traditional compounds such as trichloroacetic acid are effective protein precipitants, they are usually undesirable. The use of a single water-miscible organic solvent such as ethanol or acetone are milder, whereas a mixture of solvents (e.g., chloroform/methanol) not only denatures the protein but also effects a preliminary separation into water-soluble and organic-soluble products. Rapid freezing is a mild method of stopping reactions, but low temperature during the subsequent handling is necessary.

In toxicokinetic studies involving sequential animal sacrifice and tissue examination, it is critical to obtain uncontaminated organ samples. Apart from contamination by blood, suitable samples can be obtained by careful dissection and rinsing of the organs in ice-cold buffer, saline, or other appropriate solution. Blood samples themselves are obtained by cardiac puncture, and blood contamination of organ samples is minimized by careful bleeding of the animal at the time of sacrifice or, if necessary, by perfusion of the organ in question.

25.2.3 Forensic Studies

Because forensic toxicology deals primarily with sudden or unexpected death, the range of potential toxicants is extremely large. The analyst does not usually begin examination of the samples until all preliminary studies are complete, including autopsy and microscopic examination of all tissues. Thus the analyst is usually able to begin with some working hypothesis of the possible range of toxicants involved.

Because further sampling usually involves exhumation and is therefore unlikely or, in the case of cremation, impossible, adequate sampling and sample preservation is essential. For example, various body fluids must be collected in a proper way: blood by cardiac puncture, never from the body cavity; urine from the urinary bladder; bile collected intact as part of the ligated gallbladder; and so on. Adequate sample size is important. Blood can be analyzed for carbon monoxide, ethanol, and other alcohols, barbiturates, tranquilizers, and other drugs; at least 100 mL should be collected. Urine is useful for analysis of both endogenous and exogenous chemicals and the entire content of the bladder is retained. The liver frequently contains high levels of toxicants and/or their metabolites, and it and the kidney are the most important solid tissues for forensic analysis; 100 to 200 g of the former and the equivalent of one kidney usually are retained. DNA analysis has made tremendous strides through the use of polymerase chain reaction (PCR) that allows old samples (e.g., exhumation and sampling of bone marrow) to be analyzed and compared to living relatives; thus these data provide valuable information to law authorities and others.

An unusual requirement with important legal ramifications is that of possession. An unbroken chain of identifiable possession (i.e., chain of custody) must be maintained. All transfers are marked on the samples as to time and date of collection, arrival at the laboratory, and all transfers must be signed by both parties. The security and handling of samples during time of possession must be verifiable as a matter of law.

25.2.4 Sample Preparation

Extraction. In most cases the analysis of a pollutant or other toxicant depends on its physical removal from the sample medium. In order to ensure that the sample used is homogeneous, it is chopped, ground or blended to a uniform consistency and then subsampled. This subsample is extracted, which involves bringing a suitable solvent into intimate contact with the sample, generally in a ratio of 5 to 25 volumes of solvent to 1 volume of sample. One or more of four different procedures can be used, depending on the chemical and physical characteristics of the toxicant and the sample matrix. Other extraction methods such as boiling, grinding, or distilling the sample with appropriate solvents are used less frequently.

Blending. The use of an electric or air-driven blender is currently the most common method of extraction of biologic materials. The weighed sample is placed in a container, solvent is added, and the tissue is homogenized by motor-driven blades. Blending for 5 to 15 minutes followed by a repeat blending will extract most environmental toxicants. A homogenate in an organic solvent can be filtered through anhydrous sodium sulfate to remove water that might cause problems in the quantitation phase of the analysis. The use of sonication is a popular method for extracting tissue samples, particularly when the binding of toxicants to subcellular fractions is of interest. Sonicator probes rupture cells rapidly, thus allowing the solvent to come into intimate contact with all cell components. Differential centrifugation can then be used to isolate fractions of interest. Large wattage (e.g., 450 watt) sonifiers are used to extract compounds from environmental samples, and several US EPA methods list sonification as a valid method of extraction.

Shaking. Pollutants are generally extracted from water samples, and in some cases soil samples, by shaking with an appropriate solvent or solvent combination. Mechanical shakers are used to handle several water or soil samples at once. These devices allow the analyst to conduct long-term extractions (e.g., 24 h) if required. Two or more shakings normally are required for complete removal (i.e., >98%) of the toxicant from the sample matrix.

Washing. Washing with water-detergent combinations or with solvents can be used to remove surface contamination from environmental samples such as fruits or plants or from a worker's hands, if dermal exposure from industrial chemicals or pesticides is suspected.

Continuous Extraction. The procedure, called Soxhlet extraction, is performed on solid samples (e.g., soil) and involves the use of an organic solvent or combination of solvents. The sample is weighed into a cup (thimble) of specialized porous material such as cellulose or fiberglass and placed in the apparatus. This consists of a boiling flask, in which the solvent is placed: an extractor, which holds the thimble, and a water-jacketed condenser. When heated to boiling, the solvent vaporizes, is condensed, and fills the extractor, thus bathing the sample and extracting the toxicant. A siphoning action drains the solvent back into the boiling flask, and the cycle begins again. Depending on the nature of the toxicant and sample matrix, the extraction can be completed in as little as 2 hours but may take as long as 3 to 4 days. Automated instruments have been introduced that perform the same operation in a shorter period of time (e.g., 30 min) and use much less solvent (e.g., 15–30 mL compared to 250 mL). They are expensive compared to the older method but are cost effective.

Supercritical Fluid Extraction. Conditions can be generated that allow materials to behave differently from their native state. For example, boiling points are defined as that temperature at which a liquid changes to a gas. If the liquid is contained and pressure exerted, the boiling point changes. For a particular liquid, a combination of pressure and temperature will be reached, called the critical point, at which the material is neither a liquid nor a gas. Above this point exists a region, called the supercritical region, at which increases in both pressure and temperature will have no effect on the material (i.e., it will neither condense nor boil). This so-called supercritical fluid will exhibit properties of both a liquid and a gas. The supercritical fluid penetrates materials as if it were a gas and has solvent properties like a liquid.

Of all the materials available for use as a supercritical fluid, CO_2 has become the material of choice because of its chemical properties. Instruments have been developed to utilize the principles described to effect extractions of compounds from a variety of sample matrices including asphalt, plant material, and soils (Figure 25.1). The supercritical fluid is pumped through the sample, through a filter or column to a trap where the fluid vaporizes and solvent is added to transfer the analyses to a vial for analysis. More recent instruments combine the supercritical fluid extraction system with a variety of columns and detectors to acquire data from complex samples.

25.2.5 Separation and Identification

During extraction processes, many undesirable compounds are also released from the sample matrix; these must be removed to obtain quantitative results from certain

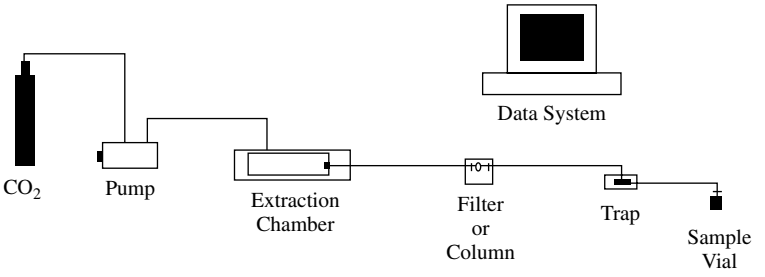


Figure 25.1 Supercritical fluid extraction.

instruments. These components include plant and animal pigments, lipids, organic material from soil and water, and inorganic compounds. If not removed, the impurities decrease the sensitivity of the detectors and columns in the analytical instrument, mask peaks, or produce extraneous peaks on chromatograms. Although some more recently developed instruments automatically remove these substances and concentrate the samples to small volumes for quantitative analysis, they are expensive. Thus most laboratories rely on other methods. These include adsorption chromatography, thin-layer chromatography (TLC), and solvent partitioning. Generally, adsorption chromatography is the method of choice to remove co-extractives from the compound in question.

Because most techniques use large volumes of solvent, the solvent must be removed to obtain a working volume (e.g., 5–10 mL) that is easy to manipulate by the analyst. This is accomplished by distillation, evaporation under a stream of air or an inert gas such as nitrogen, or evaporation under reduced pressure. Once the working volume is reached, extracts can be further purified by one or more procedures. In addition to the use of adsorbents, many organic toxicants will distribute between two immiscible solvents (e.g., chloroform and water or hexane and acetonitrile). When shaken in a separatory funnel and then allowed to equilibrate into two original solvent layers, some of the toxicant will have transferred from the original extracting solvent into the other layer. With repeated additions (e.g., 4 to 5 volumes), mixing, and removal, most or all of the compound of interest will have been transferred, leaving many interfering compounds in the original solvent. Regardless of the separation method or combination of methods used, the toxicant will be in a large volume of solvent in relation to its amount that is removed as described. Final volumes used to identify and quantitate compounds generally range from 250 μL to 10.0 mL.

Recent advances in circuit miniaturization and column technology, the development of microprocessors and new concepts in instrument design have allowed sensitive measurement at the parts per billion and parts per trillion levels for many toxicants. This increased sensitivity has focused public attention on the extent of environmental pollution, because many toxic materials present in minute quantities could not be detected until technological advances reached the present state of the art. At present, most pollutants are identified and quantified by chromatography, spectroscopy, and bioassays.

Once the toxicant has been extracted and separated from extraneous materials, the actual identification procedure can begin, although it should be remembered that the purification procedures are themselves often used in identification (e.g., peak position

in gas-liquid chromatography [GLC] and high-performance liquid chromatography [HPLC]). Thus no definite line can be drawn between the two procedures.

Chromatography. All chromatographic processes, such as TLC, GLC, HPLC, or capillary electrophoresis (CE), use a mobile and immobile phase to effect a separation of components. In TLC, the immobile phase is a thin layer of adsorbent placed on glass, resistant plastic, or fiberglass, and the mobile phase is the solvent. The mobile phase can be a liquid or gas, whereas the immobile phase can be a liquid or solid. Chromatographic separations are based on the interactions of these phases or surfaces. All chromatographic procedures use the differential distribution or partitioning of one or more components between the phases, based on the absorption, adsorption, ion-exchange, or size exclusion properties of one of the phases.

Paper Chromatography. When the introduction of paper chromatography to common laboratory use occurred in the mid-1930s, it revolutionized experimental biochemistry and toxicology. This technique is still used in laboratories that lack the expensive instruments necessary for GLC or HPLC. The stationary phase is represented by the aqueous constituent of the solvent system, which is adsorbed onto the paper; the moving phase is the organic constituents. Separation is effected by partition between the two phases as the solvent system moves over the paper. Although many variations exist, including reverse-phase paper chromatography in which the paper is treated with a hydrophobic material, ion-exchange cellulose paper, and so on, all have been superseded by equivalent systems involving thin layers of adsorbents bonded to an inert backing.

Thin-Layer Chromatography. Many toxicants and their metabolites can be separated from interfering substances with TLC. In this form of chromatography, the adsorbent is spread as a thin layer (250–2000 μm) on glass, resistant plastic or fiberglass backings. When the extract is placed near the bottom of the plate and the plate is placed in a tank containing a solvent system, the solvent migrates up the plate, and the toxicant and other constituent move with the solvent; differential rates of movement result in separation. The compounds can be scraped from the plate and eluted from the adsorbent with suitable solvents. Recent developments in TLC adsorbents allow toxicants and other materials to be quantitated at the nanogram (10^{-9} g) and picogram (10^{-12} g) levels.

Column: Adsorption, Hydrophobic, Ion Exchange. A large number of adsorbents are available to the analyst. The adsorbent can be activated charcoal, aluminum oxide, Florisil, silica, silicic acid, or mixed adsorbents. The characteristics of the toxicant determine the choice of adsorbent. When choosing an adsorbent, select conditions that either bind the co-extractives to it, allowing the compound of interest to elute, and vice versa. The efficiency of separation depends on the flow rate of solvent through the column (cartridge) and the capacity of the adsorbent to handle the extract placed on it. This amount depends on the type and quantity of adsorbent, the capacity factor (k') and concentration of sample components, and the type and strength of the solvents used to elute the compound of interest. Many environmental samples contain a sufficient amount of interfering materials so that the analyst must prepare a column using a glass chromatography tube into which the adsorbent is added. In the most common

sequence the column is packed in an organic solvent of low polarity, the sample is added in the same solvent, and the column is then developed with a sequence of solvents or solvent mixtures of increasing polarity. Such a sequence might include (in order of increasing polarity) hexane, benzene, chloroform, acetone, and methanol. Once removed, the eluate containing the toxicant is reduced to a small volume for quantitation.

However, cartridge technologies are improving to allow similar concentrations of sample to be added that result in a less expensive and more rapid analysis. A number of miniaturized columns have been introduced since the early 1980s. Most contain 0.5 to 2.0 g of the adsorbent in a plastic tube with fitted ends. The columns can be attached to standard Luer Lock syringes. Other companies have designed vacuum manifolds that hold the collecting device. The column is placed on the apparatus, a vacuum is applied, and the solvent is drawn through the column. Some advantages of these systems include preweighed amounts of adsorbent for uniformity, easy disposal of the co-extractives remaining in the cartridge, no breakage and decreased cost of the analysis because less solvent and adsorbent are used. Other forms of column chromatography can be used. They include ion-exchange chromatography, permeation chromatography, and affinity chromatography. Ion-exchange chromatography depends on the attraction between charged molecules and opposite charges on the ion exchanger, usually a resin. Compounds so bound are eluted by changes in pH and, because the net charge depends on the relationship between pH of the solution and the isoelectric point of the compounds, compounds of different isoelectric point can be eluted sequentially. Both ionic and anionic exchangers are available. Permeation chromatography utilizes the molecular sieve properties of porous materials. Molecules large enough to be excluded from the pores of the porous material will move through the column faster than will smaller molecules not excluded, thus separating them. Cross-linked dextrans such as Sephadex or agarose (Sepharose) are commonly used materials. Affinity chromatography is a potent tool for biologically active macromolecules but is seldom used for purifying small molecules, such as most toxicants. It depends on the affinity of an enzyme for a substrate (or substrate analogue) that has been incorporated into a column matrix or the affinity of a receptor for a ligand.

Gas-Liquid Chromatography (GLC). GLC is used most commonly for the separation and quantitation of organic toxicants. This system consists of an injector port, oven, detector, amplifier (electrometer), and supporting electronics (Figure 25.2). Current modern gas chromatographs use a capillary column to effect separation of complex mixtures of organic molecules and has replaced, to a large extent, the "packed" column. Instead of coating an inert support, the stationary phase is coated onto the inside of the column. The mobile phase is an inert gas (called the carrier gas), usually helium or nitrogen that passes through the column.

When a sample is injected, the injector port is at a temperature sufficient to vaporize the sample components. Based on the solubility and volatility of these components with respect to the stationary phase, the components separate and are swept through the column by the carrier gas to a detector, which responds to the concentration of each component. The detector might not respond to all components. The electronic signal produced as the component passes through the detector is amplified by the electrometer, and the resulting signal is sent to a recorder, computer, or electronic data-collecting device for quantitation.

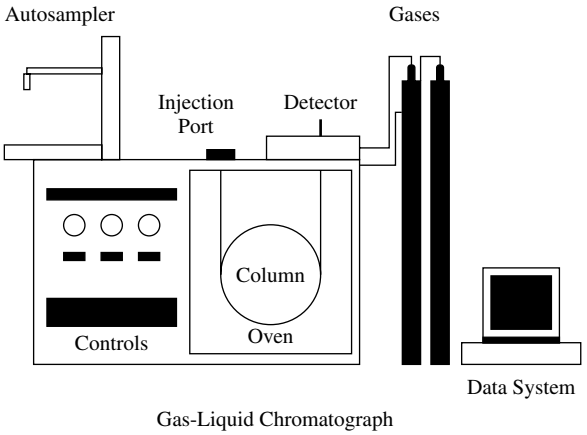


Figure 25.2 Gas-liquid chromatograph.

Column Technology. Increased sensitivity and component resolution have resulted from advances in solid-state electronics and column and detector technologies. In the field of column technology, the capillary column has revolutionized toxicant detection in complex samples. This column generally is made of fused silica 5 to 60 m in length with a very narrow inner diameter (0.23–0.75 mm) to which a thin layer (e.g., 1.0 μm) of polymer is bonded. The polymer acts as the immobile or stationary phase. The carrier gas flows through the column at flow rates of l to 2 ml/min.

Two types of capillary columns are used: the support-coated, open tubular (SCOT) column and the wall-coated, open tubular (WCOT) column. The SCOT column has a very fine layer of diatomaceous earth coated with liquid phase, that is deposited on the inside wall. The WCOT column is pretreated and then coated with a thin film of liquid phase. Of the two columns, the SCOT is claimed to be more universally applicable because of large sample capacity, simplicity in connecting it to the chromatograph, and lower cost. However, for difficult separations or highly complex mixtures, the WCOT is more efficient and is used to a much greater extent. Many older chromatographs are not designed to accommodate capillary columns, and because of these design restrictions, manufacturers offer the wide-bore capillary column along with the fittings and valving required to adapt the columns to older instruments. These columns also can be used on current instruments. With inner diameters of 0.55 to 0.75 mm, flow rates of 5.0 to 10.0 ml/min of carrier gas can be used to affect separations of components approaching that of the narrow-bore columns. Water samples chromatographed on capillary columns routinely separate 400 to 500 compounds, as compared with 90 to 120 resolved compounds from the packed column.

Detector Technology. The second advance in GLC is detector technology. Five detectors are used widely in toxicant detection: the flame ionization (FID), flame photometric (FPD), electron capture (ECD), conductivity, and nitrogen-phosphorous detectors. Other detectors have application to toxicant analysis and include the Hall conductivity detector and the photoionization detector.

The FID operates on the principle of ion formation from compounds being burned in a hydrogen flame as they elute from a column. The concentrations of ions formed

are several orders of magnitude greater than those formed in the uncontaminated flame. The ions cause a current to flow between two electrodes held at a constant potential, thus sending a signal to the electrometer.

The FPD is a specific detector in that it detects either phosphorous- or sulfur-containing compounds. When atoms of a given element are burned in a hydrogen-rich flame, the excitation energy supplied to these atoms produces a unique emission spectrum. The intensity of the wavelengths of light emitted by these atoms is directly proportional to the number of atoms excited. Larger concentrations cause a greater number of atoms to reach the excitation energy level, thus increasing the intensity of the emission spectrum. The change in intensity is detected by a photomultiplier, amplified by the electrometer, and recorded. Filters that allow only the emission wavelength of phosphorous (526 nm) or sulfur (394 nm) are inserted between the flame and the photomultiplier to give this detector its specificity.

The ECD is used to detect halogen-containing compounds, although it will produce a response to any electronegative compound. When a negative DC voltage is applied to a radioactive source (e.g., ^{63}Ni, ^{3}H), low-energy β particles are emitted, producing secondary electrons by ionizing the carrier gas as it passes through the detector. The secondary electron stream flows from the source (cathode) to a collector (anode), where the amount of current generated (called a standing current) is amplified and recorded. As electronegative compounds pass from the column into the detector, electrons are removed or "captured," and the standing current is reduced. The reduction is related to both the concentration and electronegativity of the compound passing through, and this produces a response that is recorded. The sensitivity of ECD is greater than that of any other detectors currently available.

Early electrolytic conductivity detectors operated on the principle of component combustion, which produced simple molecular species that readily ionized, thus altering the conductivity of deionized water. The changes were monitored by a dc bridge circuit and recorded. By varying the conditions, the detector could be made selective for different types of compounds (e.g., chlorine containing, nitrogen containing).

The alkali flame detector can also be made selective. Enhanced response to compounds containing arsenic, boron, halogen, nitrogen and phosphorous results when the collector (cathode) of an FID is coated with different alkali metal salts such as KBr, KCl, Na_2SO_4. As with conductivity detectors, by varying gas flow rates, types of salt, and electrode configuration, enhanced responses are obtained. The nitrogen-phosphorous alkali detector is used widely for analysis of herbicides. Alkali salts are embedded in a silica gel matrix and are heated electrically. The detector allows routine use of chlorinated solvents and derivatizing reagents that can be detrimental to other detectors.

The Hall electrolytic conductivity uses advanced designs in the conductivity cell, furnace, and an ac conductivity bridge to detect chlorine, nitrogen, and sulfur-containing compounds at sensitivities of 0.01 ng. It operates on the conductivity principle described previously. Another detector, the photoionization detector, uses an ultraviolet (UV) light source to ionize molecules by absorption of a photon of UV light. The ion formed has an energy greater than the ionization potential of the parent compound, and the formed ions are collected by an electrode. The current, which is proportional to concentration, is amplified and recorded. The detector can measure a number of organic and inorganic compounds in air, biologic fluids, and water. A number of instrument manufacturers have introduced portable GLCs that can be transported for use on field sites.

High-Performance Liquid Chromatography (HPLC). HPLC has become very popular in the field of analytical chemistry for the following reasons: it can be run at ambient temperatures; it is nondestructive to the compounds of interest, which can be collected intact; in many instances, derivatization is not necessary for response; and columns can be loaded with large quantities of the material for detection of low levels.

The instrument consists of a solvent reservoir, gradient-forming device. high-pressure pumping device, injector, column, and detector (Figure 25.3). The principle of operation is very similar to that of GLC except that the mobile phase is a liquid instead of a gas. The composition of the mobile phase and its flow rate effect separations. The columns being developed for HPLC are too numerous to discuss in detail. Most use finely divided packing (3–10 μm in diameter), some have bonded phases and others are packed with alumina or silica. The columns normally are 15 to 25 cm in length, with small diameters. (ca. 4.6 mm number diameter). A high-pressure pump is required to fi the solvent through this type of column. The major detectors presently used for HPLC are UV or fluorescent spectrophotometers or differential refractometers.

Capillary Electrophoresis (CE). A relatively new analytical technique, CE, is receiving considerable attention in the field of toxicology. Its uses appear endless, and methods have been developed to analyze a diversity of compounds, including DNA adducts, drugs, small aromatic compounds, and pesticides. Commercial instruments are available that are composed of an autosampler, high-voltage power supply, two buffer reservoirs, the capillary (approximately 70 cm × 75 μm in diameter) and a detector (Figure 25.4). The versatility of the process lies in the ability to separate compounds of interest by a number of modes, including affinity, charge/mass ratios, chiral compounds, hydrophobicity, and size. The theory of operation is simple. Because the capillary is composed of silica, silanol groups are exposed in the internal surface, which can become ionized as the pH of the eluting buffer is increased. The ionization attracts cations to the silica surface, and when current is applied, these cations migrate toward the cathode, which causes a fluid migration through the capillary. This flow can be adjusted by changing the dielectric strength of the buffer, altering the pH, adjusting the voltage, or changing the viscosity.

Under these conditions both anions and cations are separated in a single separation, with cations eluting first. Neutral molecules (e.g., pesticides) can be separated by adding a detergent (e.g., sodium dodecyl sulfate) to the buffer, forming micelles into which

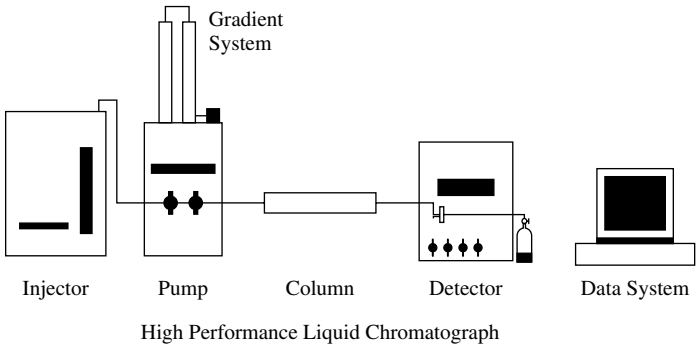


Figure 25.3 High-performance liquid chromatograph.

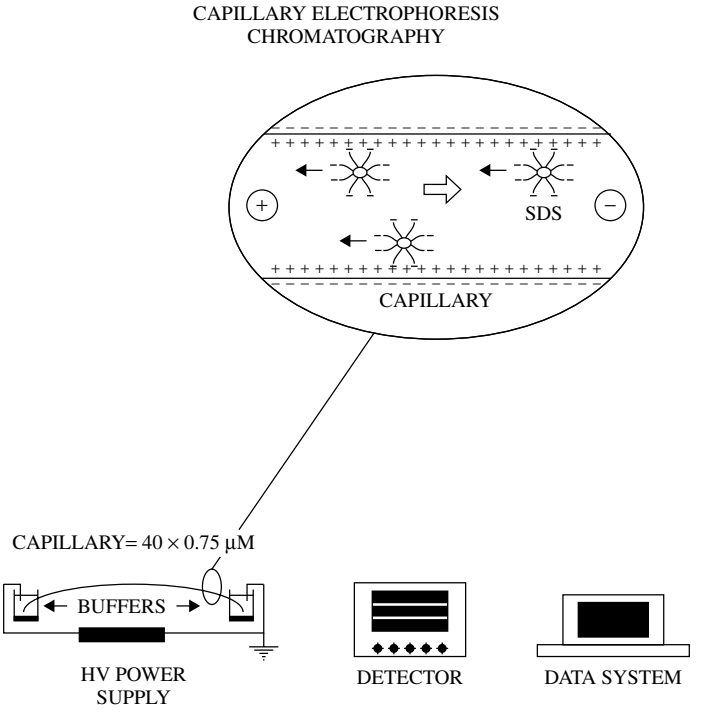


Figure 25.4 Capillary electrophoresis.

neutral molecules will partition based on their hydrophobicity. Because the micelles are attracted to the anode, they move toward the cathode at a slower rate than does the remainder of fluid in the capillary, thus allowing separation. This process is called micellar electrokinetic capillary chromatography (MECK) (Figure 25.4). Many of these analyses can be carried out in 5 to 10 minutes with sensitivities in the low parts per billion (ppb) range. A UV detector is usually used, but greatly sensitivities can be obtained using fluorescent laser detectors.

25.2.6 Spectroscopy

In certain experiments involving radiation, observed results cannot be explained on the basis of the wave theory of radiation. It must be assumed that radiation comes in discrete units, called quanta. Each quantum of energy has a definite frequency, v, and the quantum energy can be calculated by the equation $E = hv$, where h *is* Planck's constant (6.6×10^{-27}erg-s). Matter absorbs radiation one quantum at a time, and the energy of radiation absorbed becomes greater as either the frequency of radiation increases or the wavelength decreases. Therefore radiation of shorter wavelength causes more drastic changes in a molecule than does that of longer wavelength. Spectroscopy is concerned with the changes in atoms and molecules when electromagnetic radiation is absorbed or emitted. Instruments have been designed to detect these changes, and these instruments are important to the field of toxicant analysis. Discussions of atomic absorption (AA) spectroscopy, mass spectroscopy (MS), infrared (IR), and UV spectroscopy follow. A summary of spectroscopic techniques is given in Table 25.2.

Table 25.2 Characteristics of Spectroscopic Techniques

	Visible and UV spectrometry
Principle:	Energy transitions of bonding and nonbonding outerelectrons of molecules, usually delocalized electrons.
Use:	Routine qualitative and quantitative biochemical analysis including many colorimetric assays. Enzyme assays, kinetic studies, and difference spectra.
	Spectrofluorimetry
Principle:	Absorbed radiation emitted at longer wavelengths.
Use:	Routine quantitative analysis, enzyme analysis and kinetics. More sensitive at lower concentrations than visible and UV absorption.
	Infrared and Raman spectroscopy
Principle:	Atomic vibrations involving a change in dipole moment and a change in polarizability, respectively.
Use:	Qualitative analysis and fingerprinting of purified molecules of intermediate size.
	Flame spectrophotometry (emission and absorption)
Principle:	Energy transitions of outer electrons of atoms after volatilization in a flame.
Use:	Qualitative and quantitative analysis of metals; emission techniques; routine determination of alkali metals; absorption technique extends range of metals that may be determined and the sensitivity.
	Electron spin resonance
Principle:	Detection of magnetic moment associated with unpaired electrons.
Use:	Research on metalloproteins, particularly enzymes and changes in the environment of free radicals introduced into biological structures (e.g., membranes).
	Nuclear magnetic resonance
Principle:	Detection of magnetic moment associated with an odd number of protons in an atomic nucleus.
Use:	Determination of structure of organic molecules of molecular weight $< 20{,}000$ daltons.
	Mass spectrometry
Principle:	Determination of the abundance of positively ionized molecules and fragments.
Use:	Qualitative analysis of small quantities of material ($10^{-6}10^{-9}$g), particularly in conjunction with gas-liquid chromatography, HPLC and ICP.

Source: Modified from B. W. Williams and K. Wilson, *Principles and Techniques of Practical Biochemistry*, London: Edward Arnold, 1975.

Atomic Absorption Spectroscopy. One of the more sensitive instruments used to detect metal-containing toxicants is the AA spectrophotometer. Samples are vaporized either by aspiration into an acetylene flame or by carbon rod atomization in a graphite cup or tube (flameless AA). The atomic vapor formed contains free atoms of an element in their ground state, and when illuminated by a light source that radiates light of a

frequency characteristic of that element, the atom absorbs a photon of wavelength corresponding to its AA spectrum, thus exciting it. The amount of absorption is a function of concentration. The flameless instruments are much more sensitive than conventional flame AA. For example, arsenic can be detected at levels of 0.1 ng/mL and selenium at 0.2 mg/mL, which represent sensitivity three orders of magnitude greater than that of conventional flame AA.

Induced Coupled Plasma Spectrometry (ICP). An even more sensitive instrument has been developed to detect and quantitate, simultaneously, all inorganic species contained with a sample matrix. One such system is the ICP-OES (optical emission spectrometer) (Figure 25.5). The ICP-OES takes an aliquot of sample that has been acid digested and mixes it with a gas (e.g., argon) forming a plasma (i.e., an ionized gas) that is channeled into a nebulizer. Energy is applied to excite the atoms that are converted by the optics of the instrument into individual wavelengths. The

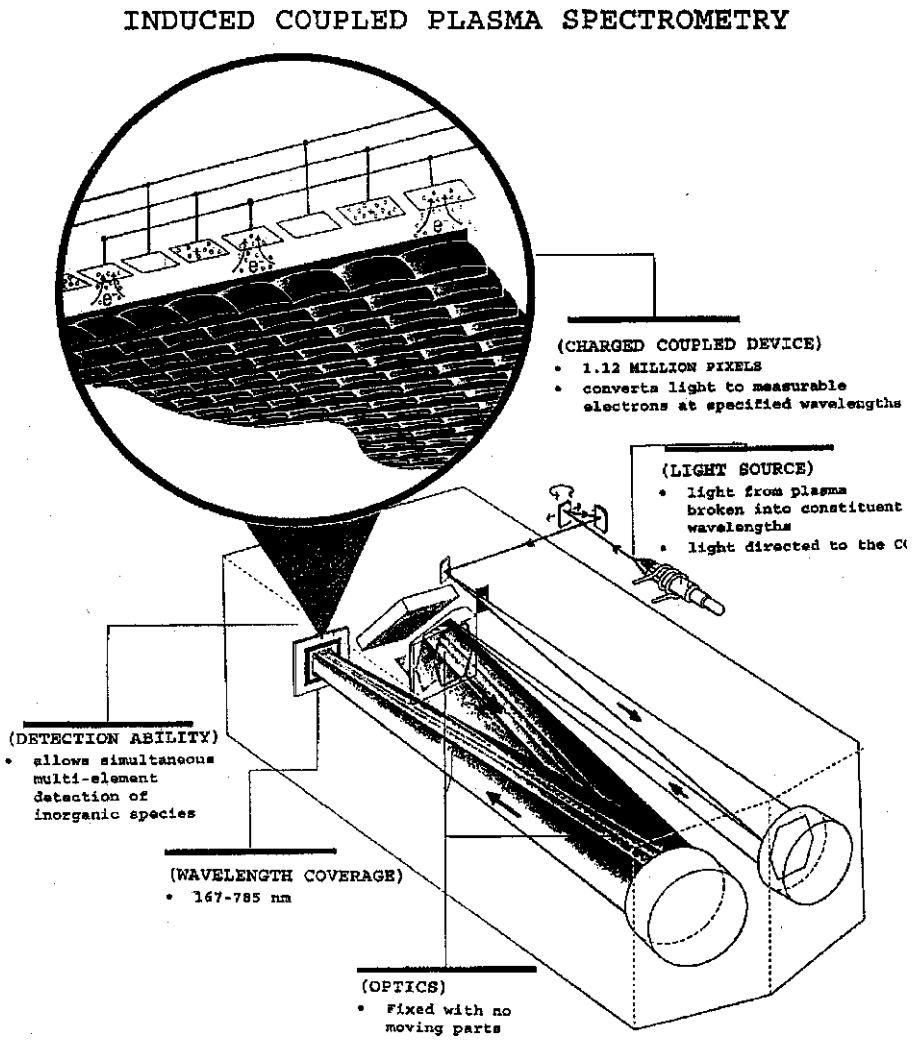


Figure 25.5 Induced coupled plasma spectrometry.

spectra are captured by a charged coupled device (CCD) that converts the light to measurable electrons at specific wavelengths. Wavelength coverage ranges from 175 to 785 nm. In addition, other instruments couple the ICP to a mass spectrometer (ICP-MS) to collect information on the analyte being sought within the sample matrix. These instruments utilize high throughput of samples and are used in both research and industrial settings.

Mass Spectroscopy (MS). The mass spectrometer is an outstanding instrument for the identification of compounds (Figure 25.6). In toxicant analysis, MS is widely used as a highly sensitive detection method for GLC and is increasingly used with HPLC, CE, and ICP because these instruments can be interfaced to the mass spectrometer. Chromatographic techniques (e.g., GLC, CE, HPLC) are used to separate individual components as previously described. A portion of the column effluent passes into the mass spectrometer, where it is bombarded by an electron beam. Electrons or negative groups are removed by this process, and the ions produced are accelerated. After acceleration they pass through a magnetic field, where the ion species are separated by the different curvatures of their paths under gravity. The resulting pattern is characteristic of the molecule under study. Two detectors are used primarily in pollutant analysis: the quadripole and the ion trap. Both produce reliable and reproducible data, and if routine maintenance is performed, both are reliable. Computer libraries of mass spectral data continue to expand, and data are generated rapidly with current software. Instrument costs have gone down, and tabletop instruments can be purchased for $70,000 although

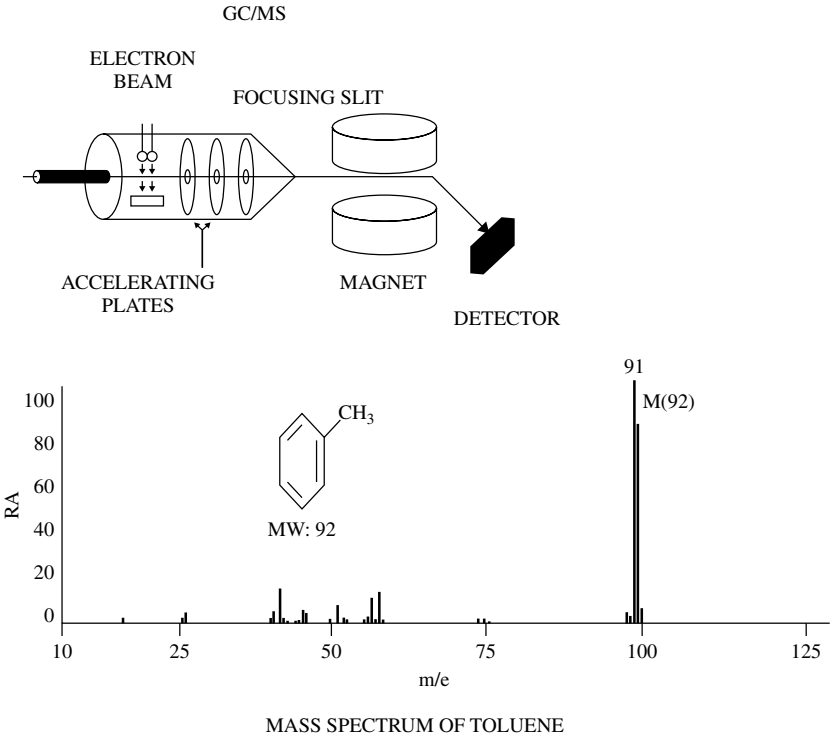


Figure 25.6 Mass spectroscopy.

research-grade instruments can cost several hundred thousand dollars. By interfacing the detector with a computer system, data reduction, analysis, and quantitation are performed automatically.

Bimolecular Interaction Analysis–Mass Spectrometry (BIA-MS). An exciting new field that is utilizing mass spectrometry as a tool in biological and toxicological research to investigate protein interactions is that of proteomics. This rapidly expanding science explores proteins within the cellular environment, their various forms, interacting partners (e.g., cofactors), and those processes that affect their regulation and processing. The BIA-MS can determine such things as the kinetics of protein interactions, selectively retrieve and concentrate specific proteins from biological media, quantitate target proteins, identify protein : ligand interactions, and recognize protein variants (e.g., point mutations). BIA-MS uses two technologies, surface plasmon resonance (SPR) sensing and matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Figure 25.7). Cells are fragmented and come in contact with a gold-plated glass slide, called a chip. The chip has highly defined sites containing a number of immobilized ligands to which the proteins of interest bind and are quantitated by SPR that monitors the interaction and quantifies the amount of protein localized at precise locations on the surface of the chip. The chip is then subjected to MALDI-TOF MS, which yields the masses of retained analytes and other bound biomolecules.

Infrared Spectrophotometry (IR). Atoms are in constant motion within molecules, and associated with these motions are molecular energy levels that correspond to the energies of quanta of IR radiation. These motions can be resolved into rotation of the whole molecule in space and into motions corresponding to the vibration of atoms with

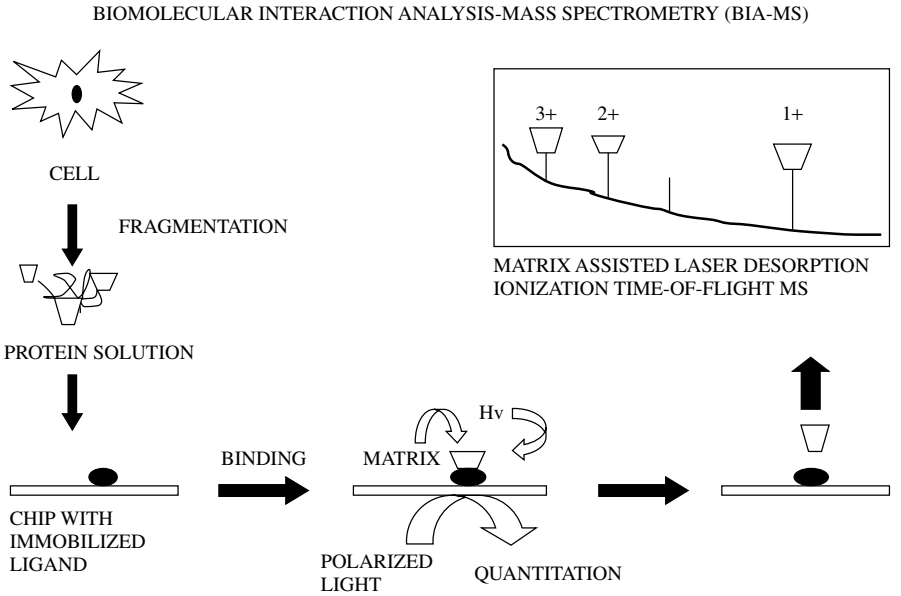


Figure 25.7 Bimolecular interaction analysis–mass spectrometry.

respect to one another by bending or stretching covalent bonds. The vibrational motions are very useful in identifying complex molecules, because functional groups (e.g., OH, C, O, SH) within the molecule have characteristic absorption bands. The principle functional groups can be determined and used to identify compounds in cases in which chemical evidence permits relatively few possible structures. Standard IR spectrophotometers cover the spectral range from 2.5 to 15.4 Nm (wave number equivalent to 4000–650 cm^{-1}) and use a source of radiation that passes through the sample and reference cells into a monochromator (a device to isolate spectral regions). The radiation is then collected, amplified, and recorded. Current instruments use microprocessors, allowing a number of refinements that have increased the versatility of IR instruments so that more precise qualitative and quantitative data can be obtained.

Ultraviolet/Visible–Spectrophotometry (UV/VIS). Transitions occur between electronic levels of molecules producing absorptions and emissions in the visible (VIS) and UV portions of the electromagnetic spectrum. Many inorganic and organic molecules show maximum absorption at specific wavelengths in the UV/VIS range, and these can be used to identify and quantitate compounds. Instruments designed to measure absorbance in the UV/VIS portions of the spectrum (190–700 nm) have been used in many specific purposes, such as detectors in HPLC and CE. These detectors use small flow cells having short path lengths (approximately 10 mm) and hold small volumes (e.g., 10.0 μL) through which light at a specific wavelength passes. Basic spectrophotometers have the same components as the IR instruments described previously, including a source (usually a deuterium lamp) monochromator, beam splitter, sampler and reference cells, and detector.

Nuclear Magnetic Resonance (NMR). Nuclear magnetic resonance (NMR) detects atoms that have nuclei and possess a magnetic moment. These are usually atoms containing nuclei with an odd number of protons (charges). Such nuclei can exist in two states: a low-energy state with the nuclear spin aligned parallel to the magnetic field and a high-energy state with the spin perpendicular to the field. Basically the instrument measures the absorption or radiowave necessary to change the nuclei from a low- to a high-energy state as the magnetic field is varied. It is used most commonly for hydrogen atoms, although ^{13}C and ^{31}P are also suitable. Because the field seen by a proton varies with its molecular environment, such molecular arrangements as CH_3, CH_2, and CH give different signs, providing much information about the structure of the molecule in question.

25.2.7 Other Analytical Methods

The instruments discussed earlier are the primary ones used in toxicant analysis, but an enormous number of analytical techniques are used in the field. Many of the instruments are expensive (e.g., Raman spectrometers, X-ray emission spectrometers) and few laboratories possess them. Many other instruments are available, however, such as the specific-ion electrode, which is both sensitive and portable. Specific-ion electrodes have many other advantages in that sample color, suspended matter, turbidity, and viscosity do not interfere with analysis; therefore many of the sample preparation steps are not required. Some of the species that can be detected at ppb levels are ammonia,

carbon dioxide, chloride, cyanide, fluoride, lead, potassium, sulfide, and urea. Analytical pH meters or meters designed specifically for this application are used to calculate concentrations.

Finally an increasing number of portable and direct reading instruments are now available to detect and quantitate environmental pollutants. Most of these measure airborne particulates and dissolved molecules and operate on such diverse principles as aerosol photometry, chemiluminescence, combustion, and polarography. Elemental analyzers have been developed for carbon, nitrogen, and sulfur using IR, chemiluminescence, and fluorescence, respectively. Analyses can be completed in about 1 minute if the samples are gases, liquids, or small solids, and within 10 minutes if solid samples are larger. These devices are microprocessor controlled, contain built-in printers, and are used to analyze materials including gasolines, pesticides, protein solutions, and wastewater.

SUGGESTED READING

Hayes, A. W., ed. *Principles and Methods of Toxicology*. London: Taylor and Francis, 2000.

Morel, F. M. M., and J. G. Heirig. *Principles and Applications of Aquatic Chemistry*. New York: Wiley-Interscience, 1993.

Nelson, A. H. *Organic Chemicals in the Aquatic Environment*. Boca Raton, FL: CRC Press, 1994.

Pickering, W. F. *Pollution Evaluation: The Quantitative Aspects*. New York: Dekker 1977.

Rand, G. M., and S. N. Petrocelli. *Fundamentals of Aquatic Toxicology*. Washington, DC: Hemisphere, 1985.

Rouessae, F., A. Rouessae, and M. B. Waldron. *Chemical Analysis: Modern Instrumentation, Methods and Techniques*. New York: Wiley-Interscience, 2000.

Thomas, J. J., P. B. Bond, and I. Sunshine, eds. *Guidelines for Analytical Toxicology Programs*, vols. 1 and 2. Cleveland: CRC Press, 1977.

Wagner, R. E. ed. *Guide to Environmental Analytical Methods*, 4th ed. Scheenectady, NY: Genium Publishing, 1998.

Ware, G. W., ed. *Reviews of Environmental Contamination and Toxicology*. New York: Springer-Verlag. (This excellent series of review articles is approaching Volume 180 and covers all aspects of toxicology.)

Williams, P. L., R. C. Jones and S. M. Roberts, eds. *The Principles of Toxicology: Environmental and Industrial Applications*, 2nd ed. New York: Wiley-Interscience, 2000.

Zweig, G. ed. *Analytical Methods for Pesticides and Plant Growth Regulators*. New York: Academic Press. (This was a multi-volume series appearing between 1973 and 1989 that contains analytical methods for the analysis of food and food additives, fungicides, herbicides, nematicides, pheromones, rodenticides, and soil fumigants.)

Web Sites

Instrument manufacturers all have detailed Web sites containing considerable information, not only on their equipment but on theory of operations, methods to maximize sensitivity, etc.

The following are some government Web sites that can be searched for analytical methods:

http://www.epa.gov/pesticides/ (A number of links to US EPA analytical methods)

http://www.nal.usda.gov/

http://npic.orst.edu/

CHAPTER 26

Basics of Environmental Toxicology

GERALD A. LEBLANC

26.1 INTRODUCTION

Industrial and agricultural endeavors are intimately associated with the extensive use of a wide array of chemicals. Historically chemical wastes generated through industrial processes were disposed of through flagrant release into the environment. Gasses quickly dispersed into the atmosphere; liquids were diluted into receiving waters and efficiently transported away from the site of generation. Similarly pesticides and other agricultural chemicals revolutionized farm and forest productivity. Potential adverse effects of the application of such chemicals to the environment were viewed as insignificant relative to the benefits bestowed by such practices. Then in 1962, a science writer for the US Fish and Wildlife Service, Rachel Carson, published a book that began by describing a world devoid of birds and from which the title *The Silent Spring* was inspired. In her book Ms. Carson graphically described incidents of massive fish and bird kills resulting from insecticide use in areas ranging from private residences to national forests. Further she inferred that such pollutant effects on wildlife may be heralding similar incipient effects on human health.

The resulting awakening of the general public to the hazards of chemicals in the environment spurred several landmark activities related to environmental protection, including Earth Day, organization of the US Environmental Protection Agency, and the enactment of several pieces of legislation aimed at regulating and limiting the release of chemicals into the environment. Appropriate regulation of the release of chemicals into the environment without applying unnecessarily stringent limitation on industry and agriculture requires a comprehensive understanding of the toxicological properties and consequences of release of the chemicals into the environment. It was from this need that modern environmental toxicology evolved.

Environmental toxicology is defined as the study of the fate and effects of chemicals in the environment. Although this definition would encompass toxic chemicals naturally found in the environment (i.e., animal venom, microbial and plant toxins), environmental toxicology is typically associated with the study of environmental chemicals of anthropogenic origin. Environmental toxicology can be divided into two subcategories:

A Textbook of Modern Toxicology, Third Edition, edited by Ernest Hodgson
ISBN 0-471-26508-X

environmental health toxicology and ecotoxicology. Environmental health toxicology is the study of the adverse effects of environmental chemicals on human health, while ecotoxicology focuses upon the effects of environmental contaminants upon ecosystems and constituents thereof (fish, wildlife, etc.). Assessing the toxic effects of chemicals on humans involves the use of standard animal models (i.e., mouse and rat) as well as epidemiological evaluations of exposed human populations (i.e., farmers and factory workers). In contrast, ecotoxicology involves the study of the adverse effects of toxicants on myriad of organisms that compose ecosystems ranging from microorganisms to top predators. Further, comprehensive insight into the effects of chemicals in the environment requires assessments ancillary to toxicology such as the fate of the chemical in the environment (Chapter 27), and toxicant interactions with abiotic (nonliving) components of ecosystems. Comprehensive assessments of the adverse effects of environmental chemicals thus utilize expertise from many scientific disciplines. The ultimate goal of these assessments is elucidating the adverse effects of chemicals that are present in the environment (retrospective hazard assessment) and predicting any adverse effects of chemicals before they are discharged into the environment (prospective hazard assessment). The ecological hazard assessment process is discussed in Chapter 28.

Historically chemicals that have posed major environmental hazards tend to share three insidious characteristics: environmental persistence, the propensity to accumulate in living things, and high toxicity.

26.2 ENVIRONMENTAL PERSISTENCE

Many abiotic and biotic processes exist in nature that function in concert to eliminate (i.e., degrade) toxic chemicals. Accordingly many chemicals released into the environment pose minimal hazard simply because of their limited life span in the environment. Chemicals that have historically posed environmental hazard (i.e., DDT, PCBs, TCDD) resist degradative processes and accordingly persist in the environment for extremely long periods of time (Table 26.1). Continued disposal of persistent chemicals into the environment can result in their accumulation to environmental levels sufficient to pose toxicity. Such chemicals can continue to pose hazard long after their disposal into the environment has ceased. For example, significant contamination of Lake Ontario by the pesticide mirex occurred from the 1950s through the 1970s. Mass balance studies performed 20 years later revealed that 80% of the mirex deposited into the lake

Table 26.1 Environmental Half-lives of Some Chemical Contaminants

Contaminant	Half-life	Media
DDT	10 Years	Soil
TCDD	9 Years	Soil
Atrazine	25 Months	Water
Benzoperylene (PAH)	14 Months	Soil
Phenanthrene (PAH)	138 Days	Soil
Carbofuran	45 Days	Water

persisted. One decade following the contamination of Lake Apopka, Florida, with pesticides including DDT and diclofol, populations of alligators continued to experience severe reproductive impairment. Both biotic and abiotic processes contribute to the degradation of chemicals.

26.2.1 Abiotic Degradation

A plethora of environmental forces compromise the structural integrity of chemicals in the environment. Many prominent abiotic degradative processes occur due to the influences of light (photolysis) and water (hydrolysis).

Photolysis. Light, primarily in the ultraviolet range, has the potential to break chemical bonds and thus can contribute significantly to the degradation of some chemicals. Photolysis is most likely to occur in the atmosphere or surface waters where light intensity is greatest. Photolysis is dependent upon both the intensity of the light and the capacity of the pollutant molecules to absorb the light. Unsaturated aromatic compounds such as the polycyclic aromatic hydrocarbons tend to be highly susceptible to photolysis due to their high capacity to absorb light energy. Light energy can also facilitate the oxygenation of environmental contaminants via hydrolytic or oxidative processes. The photooxidation of the organophosphorus pesticide parathion is depicted in Figure 26.1.

Hydrolysis. Water, often in combination with light energy or heat, can break chemical bonds. Hydrolytic reactions commonly result in the insertion of an oxygen atom into the molecule with the commensurate loss of some component of the molecule. Ester bonds, such as those found in organophosphate pesticides (i.e., parathion; Figure 26.1), are highly susceptible to hydrolysis which dramatically lowers the environmental half-lives of these chemicals. Hydrolytic rates of chemicals are influenced by the temperature and pH of the aqueous media. Rates of hydrolysis increase with increasing temperature and with extremes in pH.

26.2.2 Biotic Degradation

While many environmental contaminants are susceptible to abiotic degradative processes, such processes often occur at extremely slow rates. Environmental degradation of chemical contaminants can occur at greatly accelerated rates through the action of microorganisms. Microorganisms (primarily bacteria and fungi) degrade chemicals in an effort to derive energy from these sources. These biotic degradative processes are enzyme mediated and typically occur at rates that far exceed abiotic degradation. Biotic degradative processes can lead to complete mineralization of chemicals to water, carbon dioxide, and basic inorganic constituents. Biotic degradation includes those processes associated with abiotic degradation (i.e., hydrolysis, oxidation) and processes such as the removal of chlorine atoms (dehalogenation), the scission of ringed structures (ring cleavage), and the removal of carbon chains (dealkylation). The process by which microorganisms are used to facilitate the removal of environmental contaminants is called bioremediation.

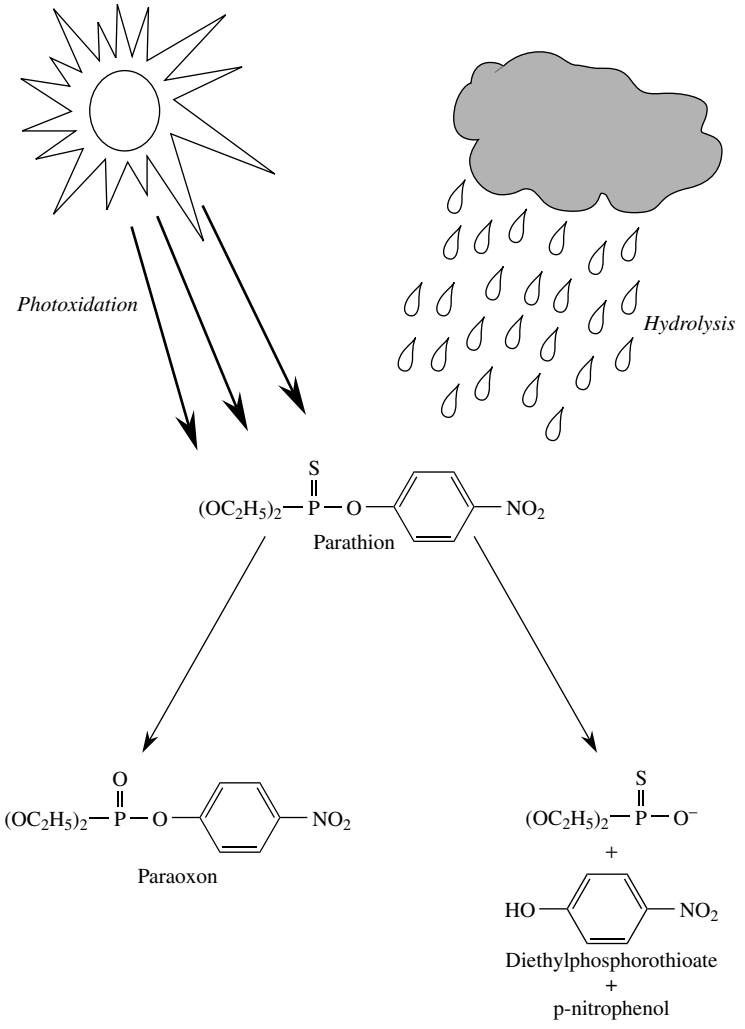


Figure 26.1 The effect of sunlight (photooxidation) and precipitation (hydrolysis) on the degradation of parathion.

26.2.3 Nondegradative Elimination Processes

Many processes are operative in the environment that contribute to the regional elimination of a contaminant by altering its distribution. Contaminants with sufficiently high vapor pressure can evaporate from contaminated terrestrial or aquatic compartments and be transferred through the atmosphere to new locations. Such processes of global distillation are considered largely responsible for the worldwide distribution of relatively volatile organochlorine pesticides such as lindane and hexachlorobenzene. Entrainment by wind and upper atmospheric currents of contaminant particles or dust onto which the contaminants are sorbed also contribute to contaminant redistribution. Sorption of contaminant to suspended solids in an aquatic environment with commensurate sedimentation can result with the removal of contaminants from the water

column and its redistribution into bottom sediments. Sediment sorption of contaminants greatly reduces bioavailability, since the propensity of a lipophilic chemical to partition from sediments to organisms is significantly less than its propensity to partition from water to organism. More highly water soluble contaminants can be removed and redistributed through runoff and soil percolation. For example, the herbicide atrazine is one of the most abundantly used pesticides in the United States. It is used to control broadleaf and weed grasses in both agriculture and landscaping. Atrazine is ubiquitous in surface waters due to its extensive use. A study of midwestern states revealed that atrazine was detectable in 92% of the reservoirs assayed. In addition atrazine has the propensity to migrate into groundwater because of its relatively high water solubility and low predilection to sorb to soil particles. Indeed, field studies have shown that surface application of atrazine typically results in the contamination of the aquifer below the application site. A more detailed account of the fate of chemicals in the environment is presented in Chapter 27.

26.3 BIOACCUMULATION

Environmental persistence alone does not render a chemical problematic in the environment. If the chemical cannot enter the body of organisms, then it would pose no threat of toxicity (see Chapter 6). Once absorbed, the chemical must accumulate in the body to sufficient levels to elicit toxicity. Bioaccumulation is defined as the process by which organisms accumulate chemicals both directly from the abiotic environment (i.e., water, air, soil) and from dietary sources (trophic transfer). Environmental chemicals are largely taken up by organisms by passive diffusion. Primary sites of uptake include membranes of the lungs, gills, and gastrointestinal tract. While integument (skin) and associated structures (scales, feathers, fur, etc.) provide a protective barrier against many environmental insults, significant dermal uptake of some chemicals can occur. Because the chemicals must traverse the lipid bilayer of membranes to enter the body, bioaccumulation potential of chemicals is positively correlated with lipid solubility (lipophilicity).

The aquatic environment is the major site at which lipophilic chemicals traverse the barrier between the abiotic environment and the biota. This is because (1) lakes, rivers, and oceans serve as sinks for these chemicals, and (2) aquatic organisms pass tremendous quantities of water across their respiratory membranes (i.e., gills) allowing for the efficient extraction of the chemicals from the water. Aquatic organisms can bioaccumulate lipophilic chemicals and attain body concentrations that are several orders of magnitude greater that the concentration of the chemical found in the environment (Table 26.2). The degree to which aquatic organisms accumulate xenobiotics from the environment is largely dependent on the lipid content of the organism, since body lipids serve as the primary site of retention of the chemicals (Figure 26.2).

Chemicals can also be transferred along food chains from prey organism to predator (trophic transfer). For highly lipophilic chemicals, this transfer can result in increasing concentrations of the chemical with each progressive link in the food chain (biomagnification). As depicted in Figure 26.3, a chemical that bioaccumulates by a factor of 2 regardless of whether the source of the contaminant is the water or food would have the potential to magnify at each trophic level leading to high levels in the birds of

Table 26.2 Bioaccumulation of Some Environmental Contaminants by Fish

Chemical	Bioaccumulation Factor[a]
DDT	127,000
TCDD	39,000
Endrin	6,800
Pentachlorobenzene	5,000
Lepthophos	750
Trichlorobenzene	183

Source: Data derived from G. A. LeBlanc, 1994, *Environ. Sci. Technol*. **28**: 154–160.

[a]Bioaccumulation factor is defined as the ratio of the chemical concentration in the fish and in the water at steady-state equilibrium.

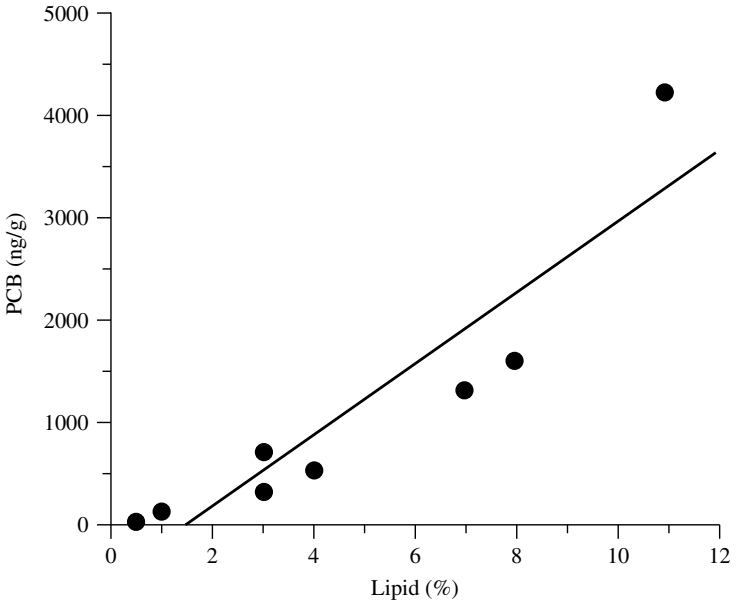


Figure 26.2 Relationship between lipid content of various organisms sampled from Lake Ontario and whole body PCB concentration (Data derived from B. G. Oliver and A. J. Niimi, *Environ. Sci. Technol*. **22**: 388–397, 1988.)

prey relative to that found in the abiotic environment. It should be noted that bioaccumulation is typically much greater from water than from food, and it is unlikely that an organism would accumulate a chemical to the same degree from both sources. The food-chain transfer of DDT was responsible for the decline in many bird-eating raptor populations that contributed to the decision to ban the use of this pesticide in the United States.

Bioaccumulation can lead to a delayed onset of toxicity, since the toxicant may be initially sequestered in lipid deposits but is mobilized to target sites of toxicity

BIOACCUMULATION OF ENVIRONMENTAL CHEMICALS

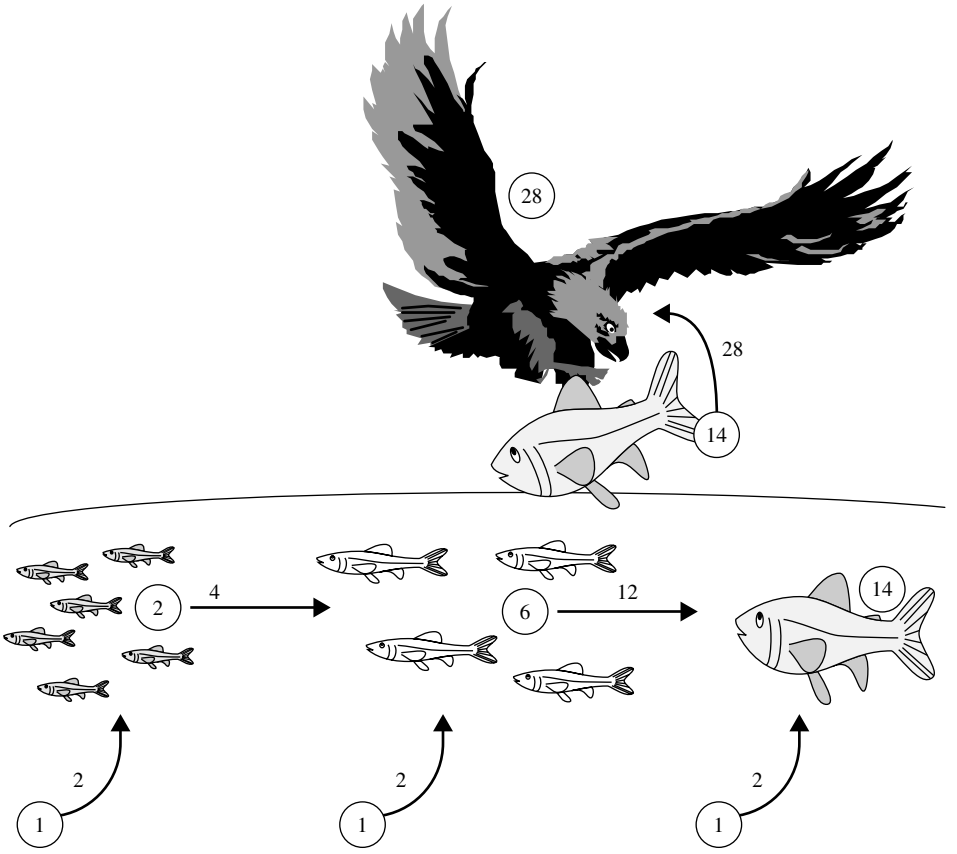


Figure 26.3 Bioaccumulation of a chemical along a generic food chain. In this simplistic paradigm, the amount of the chemical in the water is assigned an arbitrary concentration of 1, and it is assumed that the chemical will bioaccumulate either from the water to the fish or from one trophic level to another by a factor of 2. Circled numbers represent the concentration of chemical in the respective compartment. Numbers associated with arrows represent the concentration of chemical transferred from one compartment to another.

when these lipid stores are utilized. For example, lipid stores are often mobilized in preparation for reproduction. The loss of the lipid can result in the release of lipophilic toxicants rendering them available for toxic action. Such effects can result in mortality of adult organisms as they approach reproductive maturity. Lipophilic chemicals also can be transferred to offspring in lipids associated with the yolk of oviparous organisms or the milk of mammals, resulting in toxicity to offspring that was not evident in the parental organisms.

26.3.1 Factors That Influence Bioaccumulation

The propensity for an environmental contaminant to bioaccumulate is influenced by several factors. The first consideration is environmental persistence. The degree to

Table 26.3 Measured and Predicted Bioaccumulation Factors in Fish of Chemicals That Differ in Susceptibility to Biotransformation

Chemical	Susceptibility to Biotransformation	Bioaccumulation Factor	
		Predicted	Measured
Chlordane	Low	47,900	38,000
PCB	Low	36,300	42,600
Mirex	Low	21,900	18,200
Pentachloro-phenol	High	4,900	780
Tris(2,3-dibromo-propyl)phosphate	High	4,570	3

Source: Predicted bioaccumulation factors were based upon their relative lipophilicity as described by, D. Mackay, *Environ. Sci. Technol*. 1982, **16**: 274–278.

which a chemical bioaccumulates is dictated by the concentration present in the environment. Contaminants that are readily eliminated from the environment will generally not be available to bioaccumulate. An exception would be instances where the contaminant is continuously introduced into the environment (i.e., receiving water of an effluent discharge).

As discussed above, lipophilicity is a major determinant of the bioaccumulation potential of a chemical. However, lipophilic chemicals also have greater propensity to sorb to sediments, thus rendering them less available to bioaccumulate. For example, sorption of benzo[a]pyrene to humic acids reduced its propensity to bioaccumulate in sunfish by a factor of three. Fish from oligotrophic lakes, having low suspended solid levels, have been shown to accumulate more DDT than fish from eutrophic lakes that have high suspended solid contents.

Once absorbed by the organism, the fate of the contaminant will influence its bioaccumulation. Chemicals that are readily biotransformed (Chapter 7) are rendered more water soluble and less lipid soluble. The biotransformed chemical is thus less likely to be sequestered in lipid compartments and more likely to be eliminated from the body. As depicted in Table 26.3, chemicals that are susceptible to biotransformation, bioaccumulate much less than would be predicted based on lipophilicity. Conjugation of xenobiotics to glutathione and glucuronic acid (Chapter 7) can target the xenobiotic for biliary elimination through active transport processes thus greatly increasing the rate of elimination (Chapter 10). Differences in chemical elimination rates contribute to species differences in bioaccumulation.

26.4 TOXICITY

26.4.1 Acute Toxicity

Acute toxicity is defined as toxicity elicited as a result of short-term exposure to a toxicant. Incidences of acute toxicity in the environment are commonly associated with accident (i.e., derailment of a train resulting in leakage of a chemical into a river) or imprudent use of the chemical (i.e., aerial drift of a pesticide to nontarget areas). Discharge limits placed upon industrial and municipal wastes, when adhered to, have been generally successful in protecting against acute toxicity to organisms in waste-receiving areas. As discussed in Chapter 11, the acute toxicity of a chemical is commonly quantified as the LC50 or LD50. These measures do not provide any insight

Table 26.4 Ranking Scheme for Assessing the Acute Toxicity of Chemicals to Fish and Wildlife

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

into the environmentally acceptable levels of contaminants (a concentration that kills 50% of the exposed organisms is hardly tolerable). However, LC50 and LD50 values do provide statistically sound, reproducible measures of the relative acute toxicity of chemicals. LC50 and LD50 ranges for aquatic and terrestrial wildlife, respectively, and their interpretation are presented in Table 26.4.

Acute toxicity of environmental chemicals is determined experimentally with select species that serve as representatives of particular levels of trophic organization within an ecosystem (i.e., mammal, bird, fish, invertebrate, vascular plant, algae). For example, the US Environmental Protection Agency requires acute toxicity tests with representatives of at least eight different species of freshwater and marine organisms (16 tests) that include fish, invertebrates, and plants when establishing water quality criteria for a chemical. Attempts are often made to rank classes of organisms by toxicant sensitivity; however, no organism is consistently more or less susceptible to the acute toxicity of chemicals. Further the use of standard species in toxicity assessment presumes that these species are "representative" of the sensitivity of other members of that level of ecological organization. Such presumptions are often incorrect.

26.4.2 Mechanisms of Acute Toxicity

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

Cholinesterase Inhibition. The inhibition of cholinesterase activity is characteristic of acute toxicity associated with organophosphate and carbamate pesticides (see Chapter 11 for more detail on cholinesterase inhibition). Forty to 80% inhibition of brain cholinesterase activity is typically reported in lethally poisoned fish. Acute toxicity resulting from cholinesterase inhibition is relatively common among incidents of acute poisoning of fish and birds due to the high volume usage of organophosphates and carbamates in applications such as lawn care, agriculture, and golf course maintenance. Cholinesterase inhibition in fish may occur following heavy rains in aquatic habitats adjacent to areas treated with the pesticides and subject to runoff from these areas. Acute toxicity to birds commonly occurs in birds that feed in areas following application of the pesticides.

Narcosis. A common means by which industrial chemicals elicit acute toxicity, particularly to aquatic organisms, is through narcosis. Narcosis occurs when a chemical accumulates in cellular membranes interfering with the normal function of the membranes. Typical responses to the narcosis are decreased activity, reduced reaction to external stimuli, and increased pigmentation (in fish). The effects are reversible, and nonmoribund organisms typically return to normal activity once the chemical is removed from the organism's environment. Prolonged narcosis can result in death. Approximately 60% of industrial chemicals that enter the aquatic environment elicit acute toxicity through narcosis. Chemicals that elicit toxicity via narcosis typically do not elicit toxicity at specific target sites and are sufficiently lipophilic to accumulate in the lipid phase or the lipid-aqueous interface of membranes to sufficient levels to disrupt membrane function. Chemicals that induce narcosis include alcohols, ketones, benzenes, ethers, and aldehydes.

Physical Effects. Perhaps most graphic among recent incidents of environmental acute toxicity is the physical effects of petroleum following oil spills. Slicks of oil on the surface of contaminated waters results in the coating of animals, such as birds and marine mammals, that frequent the air-water interface. Such a spill of unprecedented magnitude and consequence in the United States occurred on March 24, 1989, when the hull of the Exxon Valdez was ruptured on Bligh Reef in Prince William Sound, Alaska. Nearly 11 million gallons of crude oil spilled onto the nearshore waters killing more wildlife than any prior oil spill in history. Thousands of sea birds and mammals succumbed to the acute effects of the oil.

Hypothermia is considered a major cause of death of oiled marine birds and mammals. These organisms insulate themselves from the frigid waters by maintaining a layer of air among the spaces within their coat of fur or feathers. The oil penetrates the fur/feather barrier and purges the insulating air. As a result the animals rapidly succumb to hypothermia. In addition to hypothermia, these animals can also experience oil toxicosis. Inhalation of oil, as well as ingestion through feeding and preening, can result in the accumulation of hydrocarbons to toxic levels. Toxicity to sea otters has been correlated to degree of oiling and is characterized by pulmonary emphysema (bubbles of air within the connective tissue of the lungs), gastric hemorrhages, and liver damage.

26.4.3 Chronic Toxicity

Chronic toxicity is defined as toxicity elicited as a result of long-term exposure to a toxicant. Sublethal end points are generally associated with chronic toxicity. These include reproductive, immune, endocrine, and developmental dysfunction. However, chronic exposure also can result in direct mortality not observed during acute exposure. For example, chronic exposure of highly lipophilic chemicals can result in the eventual bioaccumulation of the chemical to concentrations that are lethal to the organisms. Or as discussed previously, mobilization of lipophilic toxicants from lipid compartments during reproduction may result in lethality. It is important to recognize that, while theoretically, all chemicals elicit acute toxicity at a sufficiently high dose, all chemicals are not chronically toxic. Chronic toxicity is measured by end points such as the highest level of the chemical that does not elicit toxicity during continuous, prolonged exposure (no observed effect level, NOEL), the lowest level of the chemical that elicits

Table 26.5 Acute and Chronic Toxicity of Pesticides Measured from Laboratory Exposures of Fish Species

Pesticide	LC50 (μg/L)	Acute Toxicity	Chronic Value (μg/L)	ACR	Chronic Toxicity
Endosulfan	166	Extremely toxic	4.3	39	Yes
Chlordecone	10	Extremely toxic	0.3	33	Yes
Malathion	3,000	Very toxic	340	8.8	No
Carbaryl	15,000	Moderately toxic	378	40	Yes

toxicity during continuous, prolonged exposure (lowest observed effect level, LOEL), or the chronic value (CV) which is the geometric mean of the NOEL and the LOEL. Chronic toxicity of a chemical is often judged by the acute : chronic ratio (ACR), which is calculated by dividing the acute LC50 value by the CV. Chemicals that have an ACR of less than 10 typically have low to no chronic toxicity associated with them (Table 26.5).

The following must always be considered when assessing the chronic toxicity of a chemical: (1) Simple numerical interpretations of chronic toxicity based on ACRs serve only as gross indicators of the potential chronic toxicity of the chemical. Laboratory exposures designed to establish chronic values most often focus upon a few general endpoints such as survival, growth, and reproductive capacity. Examination of more subtle end points of chronic toxicity may reveal significantly different chronic values. (2) Laboratory exposures are conducted with a few test species that are amenable to laboratory manipulation. The establishment of chronic and ACR values with these species should not be considered absolute. Toxicants may elicit chronic toxicity in some species and not in others. (3) Interactions among abiotic and biotic components of the environment may contribute to the chronic toxicity of chemicals, while such interactions may not occur in laboratory assessments of direct chemical toxicity. These considerations are exemplified in the following incidence of chronic toxicity of chemicals in the environment.

26.4.4 Species-Specific Chronic Toxicity

Tributyltin-Induced Imposex in Neogastropods. Scientists noted in the early 1970s that dogwhelks inhabiting the coast of England exhibited a hermaphroditic-like condition whereby females possessed a penis in addition to normal female genitalia. While hermaphroditism is a reproductive strategy utilized by some molluscan species, dogwhelks are dioecious. This pseudohermaphroditic condition, called imposex, has since been documented worldwide in over 140 species of neogastropods. Imposex has been implicated in reduced fecundity of neogastropod populations, population declines, and local extinction of affected populations.

The observation that imposex occurred primarily in marinas suggested causality with some contaminant originating from such facilities. Field experiments demonstrated that neogastropods transferred from pristine sites to marinas often developed imposex. Laboratory studies eventually implicated tributyltin, a biocide used in marine paints, as the cause of imposex. Tributyltin is toxic to most marine species evaluated in the

Table 26.6 Toxicity of Tributyltin to Aquatic Organisms

Species	Acute Toxicity (LC50, μg/L)	Chronic Toxicity (LOEL, μg/L)	Imposex (μg/L)
Daphnid	1.7	—	—
Polychaete worm	—	0.10	—
Copepod	1.0	0.023	—
Oyster	1.3	0.25	—
Dogwhelk	—	—	≤0.0010

laboratory at low parts-per-billion concentrations (Table 26.6). However, exposure of neogastropods to low parts-per-trillion concentrations can cause imposex (Table 26.6). Thus neogastropods are uniquely sensitive to the toxicity of tributyltin, with effects produced that were not evident in standard laboratory toxicity characterizations.

Atrazine-Induced Hermaphroditism in Frogs. The herbicide atrazine historically has been considered environmentally safe for use since the material has proved to be only slightly to moderately toxic in standard fish and wildlife toxicity evaluations. Measured atrazine levels in surface waters rarely exceed 0.04 mg/L. The acute and chronic toxicities of atrazine to aquatic organisms are typically in excess of 1 mg/L. Thus ample safety margins appear to exist for this compound. Recent studies with frogs have revealed that exposure to 0.0001 mg/L atrazine through the period of larval development caused the frogs to develop both a testis and an ovary. The toxicological significance of this chemical-induced hermaphroditic condition is not known. However, environmentally relevant levels of the herbicide appear to have the potential to adversely impact reproductive success of these organisms.

26.4.5 Abiotic and Biotic Interactions

Chlorofluorocarbons–Ozone–UV-B Radiation–Amphibian Interactions. The atmospheric release of chlorofluorocarbons has been implicated in the depletion of the earth's stratospheric ozone layer which serves as a filter against harmful ultraviolet radiation. Temporal increases in UV-B radiation have been documented and pose increasing risks of a variety of maladies to both plant and animal life.

Commensurate with the increase in UV-B radiation levels at the earth's surface has been the decline in many amphibian populations. Multiple causes may be responsible for these declines including loss of habitat, pollutants, and increased incidence of disease; however, recent studies suggest that increases in UV-B radiation may be a major contributor to the decline in some populations. Field surveys in the Cascade Mountains, Oregon, revealed a high incidence of mortality among embryos of the Cascades frog and western toad. Incubation of eggs, collected from the environment, in the laboratory along with the pond water in which the eggs were collected resulted in low mortality, suggesting that contaminants in the water were not directly responsible for the mortality. Furthermore placement of UV-B filters over the embryos, incubated under ambient environmental conditions, significantly increased viability of the embryos.

Several amphibian species were examined for photolyase activity. This enzyme is responsible for the repair of DNA damage caused by UV-B radiation. A more than

80-fold difference in photolyase activity was observed among the species examined. Photolyase activity was appreciably lower in species known to be experiencing population decline as compared to species showing stable population levels. Recent studies have also suggested that ambient UV-B radiation levels can enhance the susceptibility of amphibian embryos to mortality originating from fungal infection.

These observations suggest that chlorofluorocarbons may be contributing to the decline in amphibian populations. However, this toxicological effect is the result of abiotic interactions (i.e., chlorofluorocarbons depleting atmospheric ozone levels, which increase UV-B radiation penetration resulting in embryo mortality) (Figure 26.4). In addition abiotic (UV-B) and biotic (fungus) interactions may also be contributing to the toxicity. Such effects would not be predicted from direct laboratory assessments of the toxicity of chlorofluorocarbons to amphibians and highlight the necessity to consider possible indirect toxicity associated with environmental contaminants.

Masculinization of Fish due to Microbial Interactions with Kraft Pulpmill Effluent. Field surveys of mosquito fish populations in the state of Florida revealed populations containing females that exhibited male traits such as male-type mating behavior and the modification of the anal fin to resemble the sperm-transmitting gonopodium of males. Masculinized females were found to occur downstream of kraft pulpmill effluents suggesting that components of the effluent were responsible for the masculinizing effect. Direct toxicity assays performed with the effluent did not produce such effects. However, the inclusion of microorganisms along with the effluent resulted in masculinization. Further studies revealed that phytosterols present in the kraft pulpmill effluent can be converted to androgenic C19 steroids by microorganisms and these steroids are capable of masculinizing female fish (Figure 26.5).

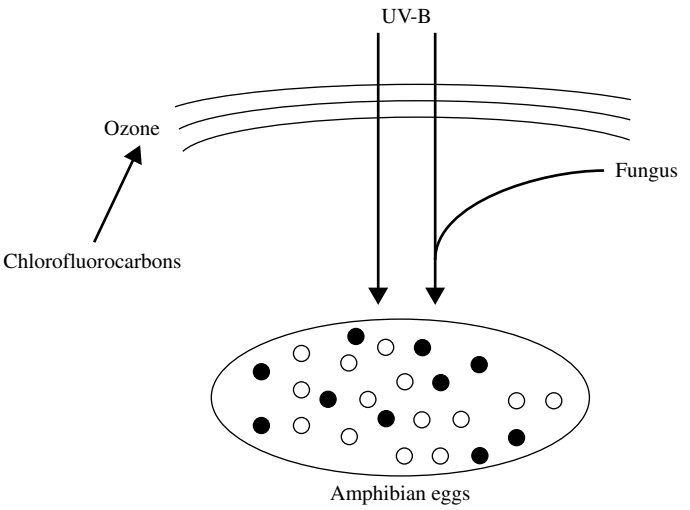


Figure 26.4 Abiotic and biotic interactions leading to the indirect toxicity of chlorofluorocarbons to amphibians. Atmospheric release of chlorofluorocarbons causes the depletion of the stratospheric ozone layer (abiotic-abiotic interaction). Depleted ozone allows for increased penetration of UV-B radiation (abiotic-abiotic interaction). UV-B radiation alone and in combination with fungus (abiotic-biotic interaction) causes increased mortality of amphibian embryos.

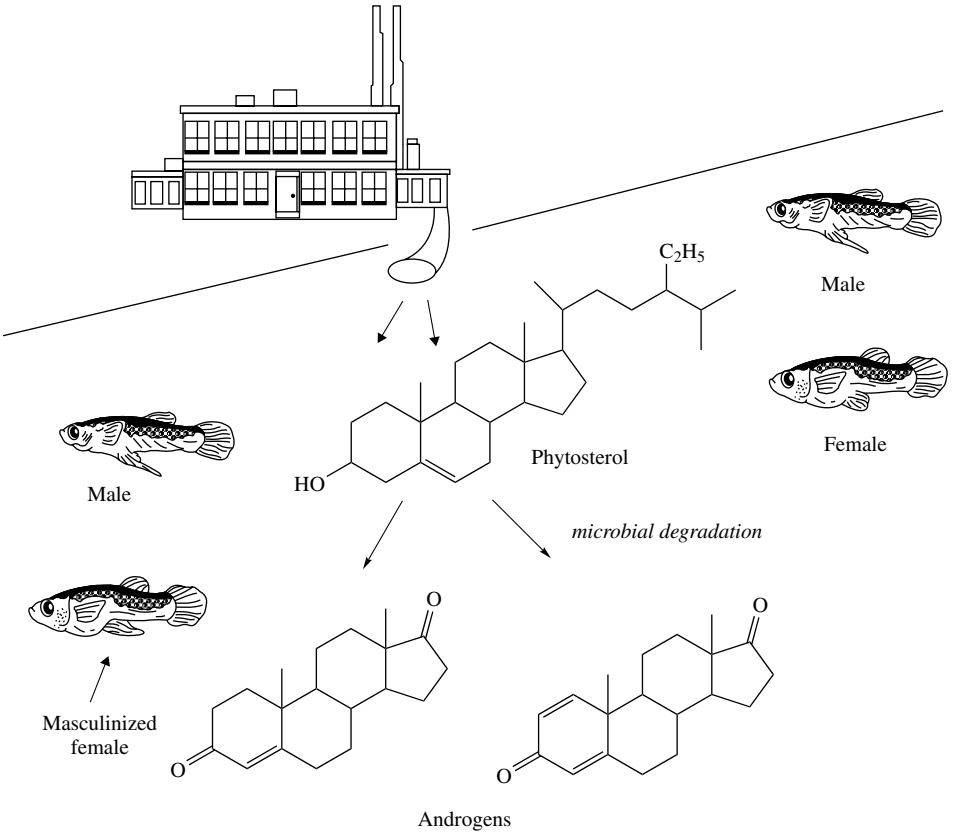


Figure 26.5 Indirect toxicity of kraft pulpmill effluent to mosquito fish. Phytosterols in the mill effluent are converted to C19 steroidal androgens through the action of microorganisms in the environment. These androgens masculinize both anatomy and behavior of female mosquito fish. An arrow identifies the modified anal fin on the masculinized female.

Thus abiotic (phytosterols) : biotic (microorganisms) interactions in the environment must occur before this occult toxicity associated with the kraft pulpmill effluent is unveiled.

Environmental Contaminants and Disease among Marine Mammals. Massive mortality have occurred over the past 20 years among populations of harbor seals, bottlenose dolphins, and other marine mammals worldwide. In many instances this mortality has been attributed to disease. For example, nearly 18,000 harbor seals died in the North, Irish, and Baltic seas in the late 1980s due to phocine distemper virus. Incidences of the disease outbreak were highest in areas containing high levels of pollutants, and seals that succumbed to the disease were found to have high tissue levels of polychlorinated biphenyls (PCBs). PCBs and other organochlorine chemicals such as DDT, hexachlorobenzene, and dieldrin have been shown to immunosuppress laboratory animals, and accumulation of these chemicals by the seals may have increased their susceptibility to the virus. This hypothesis was tested by feeding fish, caught either from a relatively pristine area or from a polluted coastal area, to seals for 93 weeks then

assessing the integrity of the immune system in the seals. Seals fed the contaminated fish did indeed have impaired immune responses lending credence to the hypothesis that organochlorine contaminants in the marine environment are rendering some species immunodeficient. Mortality occurs, not as a direct result of chemical toxicity, but due to increased susceptibility to pathogens.

26.5 CONCLUSION

Environmental toxicologists have learned a great deal about the effects of chemicals in the environment and the characteristics of chemicals that are responsible for the hazards they pose. Much of the information gained has been due to retrospective analyzes of the environmental consequences of the deposition of chemicals into the environment. Such analyzes have resulted in curtailing the release of demonstrated hazardous chemicals into the environment and provide benchmark information upon which the regulation of chemicals proposed for release into the environment can be based. The recognition that environmentally hazardous chemicals commonly share characteristics of persistence, potential to bioaccumulate, and high toxicity has resulted in development and use of chemicals that lack one or more of these characteristics yet fulfill societal needs previously served by hazardous chemicals. For example, recognition that persistence and propensity to bioaccumulate were largely responsible for the environmental hazards posed by many organochlorine pesticides led to the development and use of alternative classes of pesticides such as organophosphates, carbamates, and pyrethroids. While these chemicals all possess the toxicity necessary to function as pesticides, their lack of persistence and reduced propensity to bioaccumulate makes them more suitable for use in the environment.

Such advances in our understanding of the fate and effects of chemicals in the environmental does not imply that the role of environmental toxicologists in the twenty-first century will diminish. A dearth of information persist in areas vital to continued protection of natural resources against chemical insult. These include understanding (1) the unique susceptibilities of key species to the toxicity of different classes of chemicals, (2) the interactions of chemical contaminants with abiotic components of the environment that lead to increased toxicity, (3) the toxicological consequences of exposure to complex chemical mixtures, and (4) the consequences of toxicant effects on individuals with respect to ecosystem viability. Additionally continued research is needed to develop molecular and cellular biomarkers of toxicant exposure and effect that could be used to predict dire consequences to ecosystem before such effects are manifested at higher levels of biological organization. The role of the environmental toxicologist undoubtedly will increase in prospective activities aimed at reducing the risk associated with chemical contaminants in the environments before problems arise, and hopefully will decrease with respect to assessing damage caused by such environmental contaminants.

SUGGESTED READING

Persistence

Burns, L. A., and G. L. Baughman. Fate modeling. In *Fundamentals of Aquatic Toxicology*, G. M. Rand, and S. R. Petrocelli, eds. New York: Hemisphere, 1985, pp. 558–586.

Larson, R. A., and E. J. Weber. *Reaction Mechanisms in Environmental Organic Chemistry*. Boca Raton, FL: Lewis Publishers, 1994.

Bioaccumulation

Banerjee, S., and G. A. Baughman. Bioconcentration factors and lipid solubility. *Environ. Sci. Technol.* **25**: 536–539, 1991.

Barron, M. G. Bioconcentration. *Environ. Sci. Technol.* **24**: 1612–1618, 1990.

Barron, M. G. Bioaccumulation and bioconcentration in aquatic organisms. In *Handbook of Ecotoxicology*, D. J. Hoffman, B. A. Rattner, G. A. Burton Jr., and J. Cairns Jr., eds. Boca Raton, FL: Lewis Publishers, 1995, pp. 652–666.

LeBlanc, G. A. Trophic-level differences in the bioconcentration of chemicals: Implications in assessing environmental biomagnification. *Environ. Sci. Technol.* **28**: 154–160, 1995.

Acute Toxicity

Kelso, D. D., and M. Kendziorek. Alaska's response to the Exxon Valdez oil spill. *Environ. Sci. Technol.* **25**: 183–190, 1991.

Parrish, P. R. Acute toxicity tests. In *Fundamentals of Aquatic Toxicology*, G. M. Rand, and S. R. Petrocelli, eds. New York: Hemisphere, 1985, pp. 31–57.

Stansley, W. Field results using cholinesterase reactivation techniques to diagnose acute anticholinesterase poisoning in birds and fish. *Arch. Environ. Contam. Toxicol.* **25**: 315–321, 1993.

van Wezel, A. P., and A. Opperhuizen. Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms, and membrane burdens. *Crit. Rev. Toxicol.* **25**: 255–279, 1995.

Wilson, V. S., and G. A. LeBlanc. Petroleum pollution. *Rev. Toxicol.* **3**: 1–36, 1999.

Chronic Toxicity

Adams, W. J. Aquatic toxicology testing methods. In *Handbook of Ecotoxicology*, D. J. Hoffman, B. A. Rattner, G. A. Burton Jr., and J. Cairns Jr., eds. Boca Raton, FL: Lewis Publishers, 1995, pp. 25–46.

Blaustein, A. R., P. D. Hoffman, D. G. Hokit, J. M. Kiesecker, S. C. Walls, J. B. Hays. UV repair and resistance to solar UV-B in amphibian eggs: A link to population declines? *Proc. Nat. Acad. Sciences. USA* **91**: 1791–1795, 1994.

Colborn, T., and C. Clement, eds. *Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Princeton, NJ: Princeton Scientific, 1992.

LeBlanc, G. A., and L. J. Bain. Chronic toxicity of environmental contaminants: Sentinels and biomarkers. *Environ. Health Perspect.* **105**(suppl. 1): 65–80, 1997.

Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. A. Stuart, and A. Vonk. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proc. Nat. Acad. Sciences.* **99**: 5476–5480, 2002.

CHAPTER 27

Transport and Fate of Toxicants in the Environment

DAMIAN SHEA

27.1 INTRODUCTION

More than 100,000 chemicals are released into the global environment every year through their normal production, use, and disposal. To understand and predict the potential risk that this environmental contamination poses to humans and wildlife, we must couple our knowledge on the toxicity of a chemical to our knowledge on how chemicals enter into and behave in the environment. The simple box model shown in Figure 27.1 illustrates the relationship between a toxicant source, its fate in the environment, its effective exposure or dose, and resulting biological effects. A *prospective* or *predictive* assessment of a chemical hazard would begin by characterizing the source of contamination, modeling the chemical's fate to predict exposure, and using exposure/dose-response functions to predict effects (moving from left to right in Figure 27.1). A common application would be to assess the potential effects of a new waste discharge. A *retrospective* assessment would proceed in the opposite direction starting with some observed effect and reconstructing events to find a probable cause. Assuming that we have reliable dose/exposure-response functions, the key to successful use of this simple relationship is to develop a qualitative description and quantitative model of the sources and fate of toxicants in the environment.

Toxicants are released into the environment in many ways, and they can travel along many pathways during their lifetime. A toxicant present in the environment at a given point in time and space can experience three possible outcomes: it can be *stationary* and add to the toxicant inventory and exposure at that location, it can be *transported* to another location, or it can be *transformed* into another chemical species. Environmental contamination and exposure resulting from the use of a chemical is modified by the transport and transformation of the chemical in the environment. Dilution and degradation can attenuate the source emission, while processes that focus and accumulate the chemical can magnify the source emission. The actual fate of a chemical depends on the chemical's use pattern and physical-chemical properties, combined with the characteristics of the environment to which it is released.

A Textbook of Modern Toxicology, Third Edition, edited by Ernest Hodgson
ISBN 0-471-26508-X

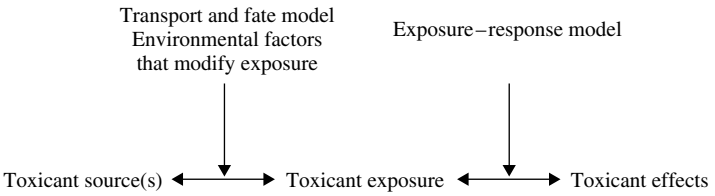


Figure 27.1 Environmental fate model. Such models are used to help determine how the environment modifies exposure resulting from various sources of toxicants.

Conceptually and mathematically, the transport and fate of a toxicant in the environment is very similar to that in a living organism. Toxicants can enter an organism or environmental system by many routes (e.g., dermal, oral, and inhalation versus smoke stack, discharge pipe, or surface runoff). Toxicants are redistributed from their point of entry by fluid dynamics (blood flow vs. water or air movement) and intermedia transport processes such as partitioning (blood-lipid partitioning vs. water-soil partitioning) and complexation (protein binding vs. binding to natural organic matter). Toxicants are transformed in both humans and the environment to other chemicals by reactions such as hydrolysis, oxidation, and reduction. Many enzymatic processes that detoxify and activate chemicals in humans are very similar to microbial biotransformation pathways in the environment.

In fact, physiologically based pharmacokinetic models are similar to environmental fate models. In both cases we divide a complicated system into simpler compartments, estimate the rate of transfer between the compartments, and estimate the rate of transformation within each compartment. The obvious difference is that environmental systems are inherently much more complex because they have more routes of entry, more compartments, more variables (each with a greater range of values), and a lack of control over these variables for systematic study. The discussion that follows is a general overview of the transport and transformation of toxicants in the environment in the context of developing qualitative and quantitative models of these processes.

27.2 SOURCES OF TOXICANTS TO THE ENVIRONMENT

Environmental sources of toxicants can be categorized as either *point sources* or *nonpoint sources* (Figure 27.2). Point sources are discrete discharges of chemicals that are usually identifiable and measurable, such as industrial or municipal effluent outfalls, chemical or petroleum spills and dumps, smokestacks and other stationary atmospheric discharges. Nonpoint sources are more diffuse inputs over large areas with no identifiable single point of entry such as agrochemical (pesticide and fertilizer) runoff, mobile sources emissions (automobiles), atmospheric deposition, desorption or leaching from very large areas (contaminated sediments or mine tailings), and groundwater inflow. Nonpoint sources often include multiple smaller point sources, such as septic tanks or automobiles, that are impractical to consider on an individual basis. Thus the identification and characterization of a source is relative to the environmental compartment or system being considered. For example, there may be dozens of important toxicant sources to a river, each must be considered when assessing the hazards of toxicants to aquatic life in the river or to humans who might drink the water or consume the

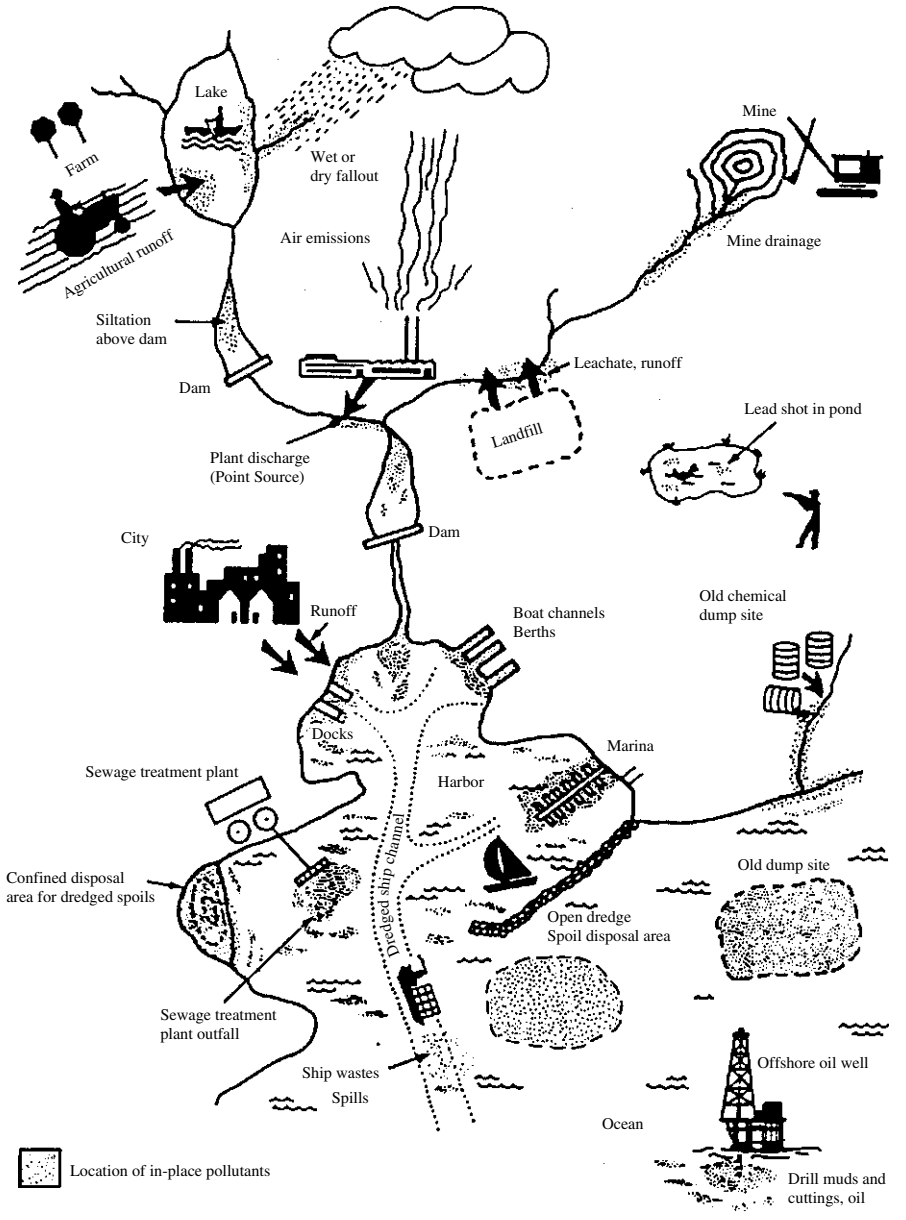


Figure 27.2 Entry of toxicants into environment through many point and nonpoint sources.

fish and shellfish. However, these toxicant sources can be well mixed in the river, resulting in a rather homogeneous and large point source to a downstream lake or estuary (Figure 27.2).

The rate (units of g/h) at which a toxicant is emitted by a source (*mass emission rate*) can be estimated from the product of the toxicant concentration in the medium (g/m^3) and the flow rate of the medium (m^3/h). This would appear to be relatively simple for point sources, particularly ones that are routinely monitored to meet environmental

regulations. However, the measurement of trace concentrations of chemicals in complex effluent matrices is not a trivial task (see Chapter 25). Often the analytical methods prescribed by environmental agencies for monitoring are not sensitive or selective enough to measure important toxicants or their reactive metabolites. Estimating the mass emission rates for nonpoint sources is usually very difficult. For example, the atmospheric deposition of toxicants to a body of water can be highly dependent on both space and time, and high annual loads can result from continuous deposition of trace concentrations that are difficult to measure. The loading of pesticides from an agricultural field to an adjacent body of water also varies with time and space as shown in Figure 27.3 for the herbicide atrazine. Rainfall following the application of atrazine results in drainage ditch loadings more than 100-fold higher than just two weeks following the rain. A much smaller, but longer lasting, increase in atrazine loading occurs at the edge of the field following the rain. Again, we see the need to define the spatial scale of concern when identifying and characterizing a source. If one is concerned with the fate of atrazine within a field, the source is defined by the application rate. If one is concerned with the fate and exposure of atrazine in an adjacent body of water, the source may be defined as the drainage ditch and/or as runoff from the edge of field. In the latter case one either needs to take appropriate measurements in the field or model the transport of atrazine from the field.

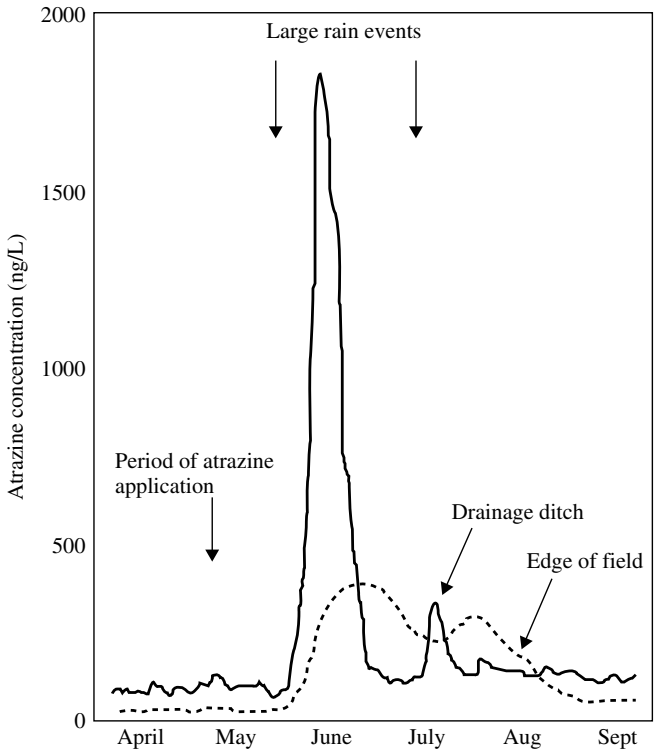


Figure 27.3 Loading of atrazine from an agricultural field to an adjacent body of water. The loading is highly dependent on rainfall and the presence of drainage ditches that collect the chemical and focus its movement in the environment.

27.3 TRANSPORT PROCESSES

Following the release of a toxicant into an environmental compartment, transport processes will determine its spatial and temporal distribution in the environment. The transport medium (or fluid) is usually either air or water, while the toxicant may be in dissolved, gaseous, condensed, or particulate phases. We can categorize physical transport as either *advection* or *diffusion*.

27.3.1 Advection

Advection is the passive movement of a chemical in bulk transport media either within the same medium (intraphase or homogeneous transport) or between different media (interphase or heterogeneous transport). Examples of homogeneous advection include transport of a chemical in air on a windy day or a chemical dissolved in water moving in a flowing stream, in surface runoff (nonpoint source), or in a discharge effluent (point source). Examples of heterogeneous advection include the deposition of a toxicant sorbed to a suspended particle that settles to bottom sediments, atmospheric deposition to soil or water, and even ingestion of contaminated particles or food by an organism (i.e., bioaccumulation). Advection takes place independently from the presence of a chemical; the chemical is simply going along for the ride. Advection is not influenced by diffusion and can transport a chemical either in the same or opposite direction as diffusion. Thus advection is often called *nondiffusive transport*.

Homogeneous Advection. The homogeneous advective transport rate (N, g/h) is simply described in mathematical terms by the product of the chemical concentration in the advecting medium (C, g/m^3) and the flow rate of the medium (G, m^3/h):

$$N = GC.$$

For example, if the flow of water out of a lake is 1000 m^3/h and the concentration of the toxicant is 1 μg/m^3, then the toxicant is being advected from the lake at a rate of 1000 μg/h (or 1 mg/h). The emission rates for many toxicant sources can be calculated in the same way.

As with source emissions, advection of air and water can vary substantially with time and space within a given environmental compartment. Advection in a stream reach might be several orders of magnitude higher during a large rain event compared to a prolonged dry period, while at one point in time, advection within a stagnant pool might be several orders of magnitude lower than a connected stream. Thus, as with source characterization, we must match our estimates of advective transport to the spatial and temporal scales of interest. Again, a good example is the movement of atrazine from an agricultural field (Figure 27.3). Peak flow advective rates that follow the rain might be appropriate for assessing acute toxicity during peak flow periods but not for estimating exposure at other times of the year. Conversely, an annual mean advective rate would underestimate exposure during peak flow but would be more appropriate for assessing chronic toxicity.

In surface waters advective currents often dominate the transport of toxicants, and they can be estimated from hydrodynamic models or current measurements. In many cases advective flow can be approximated by the volume of water exchanged per unit

time by assuming conservation of mass and measuring flow into or out of the system. This works only for well mixed systems that have no or only small volumes of stagnant water. In water bodies that experience density stratification (i.e., thermocline) separate advective models or residence times can be used for each water layer. In air, advection also dominates the transport of chemicals, with air currents being driven by pressure gradients. The direction and magnitude of air velocities are recorded continuously in many areas, and daily, seasonal, or annual means can be used to estimate advective air flow.

Advective air and water currents are much smaller in soil systems but still influence the movement of chemicals that reside in soil. Advection of water in the saturated zone is usually solved numerically from hydrodynamic models. Advection of air and water in the unsaturated zone is complicated by the heterogeneity of these soil systems. Models are usually developed for specific soil property classes, and measurements of these soil properties are made at a specific site to determine which soil-model layers to link together.

Heterogeneous Advection. Heterogeneous advective transport involves a secondary phase within the bulk advective phase, such as when a particle in air or water acts as a carrier of a chemical. In many cases we can treat heterogeneous advection the same as homogeneous advection if we know the flow rate of the secondary phase and the concentration of chemical in the secondary phase. In the lake example above, if the volume fraction of suspended particles in the lake water is 10^{-5}, the flow rate of suspended particles is 0.01 m^3/h, and the concentration of the toxicant in the solid particles is 100 mg/m^3, then the advective flow of the toxicant on suspended particles will be 1 mg/h or the same as the homogeneous advection via water. Although the flow rate of particles is much lower than that of water, the concentration of the toxicant is much higher in the suspended particles than dissolved in the water. This is typical of a hydrophobic toxicant such as DDT or benzo[a]pyrene. In soil and sedimentary systems, colloidal particles (often macromolecular detritus) can play a very important role in heterogeneous advective transport because they have greater mobility than larger particles, and they often have greater capacity to sorb many toxicants because of their higher organic carbon content and higher surface area/mass ratio. In highly contaminated sites, organic co-solvents can be present in the water (usually groundwater) and act as a high-capacity and high-efficiency carrier of toxicants through heterogeneous advection in the water.

Unfortunately, the dynamics of heterogeneous transport are rarely simple, particularly over shorter scales of time and space. In addition to advection of particles with flowing water, aqueous phase heterogeneous transport includes particle settling, resuspension, burial in bottom sediments, and mixing of bottom sediments. Particle settling can be an important mechanism for transporting hydrophobic toxicants from the water to the bottom sediments. Modeling this process can be as simple as using an overall mass transfer coefficient or can include rigorous modeling of particles with different size, density, and organic carbon content. Estimates of particle settling are usually obtained through the use of laboratory settling chambers, in situ sediment traps, or by calculation using Stoke's law. Resuspension of bottom sediments occurs when sufficient energy is transferred to the sediment bed from advecting water, internal waves, boats, dredging, fishing, and the movement of sediment dwelling organisms (i.e., bioturbation). Resuspension rates are difficult to measure and often are highly variable

in both time and space. Much as the annual runoff of pesticides from an agricultural field may be dominated by a few rain events, annual resuspension rates can be dominated by a major storm, and in smaller areas by a single boat or school of bottom fish. Resuspension rates can be estimated from sediment traps deployed just above the sediment surface or from the difference between particle settling and permanent burial or sedimentation. Sedimentation is the net result of particle settling and resuspension and can be measured using radionuclide dating methods (e.g., ^{210}Pb). Sediment dating itself becomes difficult when there is significant mixing of the surface sediments (e.g., through bioturbation). Thus the heterogeneous transport of toxicants on aqueous particles can be rather complicated, though many aquatic systems have been modeled reasonably well.

Heterogeneous advective transport in air occurs primarily through the absorption of chemicals into falling water droplets (wet deposition) or the sorption of chemicals into solid particles that fall to earth's surface (dry deposition). Under certain conditions both processes can be treated as simple first-order advective transport using a flow rate and concentration in the advecting medium. For example, wet deposition is usually characterized by a washout coefficient that is proportional to rainfall intensity.

27.3.2 Diffusion

Diffusion is the transport of a chemical by random motion due to a state of disequilibrium. For example, diffusion causes the movement of a chemical within a phase (e.g., water) from a location of relatively high concentration to a place of lower concentration until the chemical is homogeneously distributed throughout the phase. Likewise diffusive transport will drive a chemical between media (e.g., water and air) until their equilibrium concentrations are reached and thus the chemical potentials or fugacities are equal in each phase.

Diffusion within a Phase. Diffusional transport within a phase can result from random (thermal) motion of the chemical (molecular diffusion), the random turbulent mixing of the transport medium (turbulent diffusion), or a combination of both. Turbulent diffusion usually dominates the diffusive (but not necessarily the advective) chemical transport in air and water due to the turbulent motions or eddies that are common in nature. In porous media (sediment and soil) the water velocities are typically too low to create eddies, but random mixing still occurs as water tortuously flows around particles. This mechanical diffusion is often called dispersion by hydrologists and dispersion on larger scales, such as when groundwater detours around large areas of less permeable soil, is called macrodispersion. Note, however, that the term dispersion often is used by meteorologists and engineers to describe any turbulent diffusion.

Although different physical mechanisms can cause diffusive mixing, they all cause a net transport of a chemical from areas of higher concentration to areas of lower concentration. All diffusive processes are also referred to as *Fickian* transport because they all can be described mathematically by Fick's first law, which states that the flow (or flux) of a chemical (N, g/h) is proportional to its concentration gradient (dC/dx):

$$N = -DA\left(\frac{dC}{dx}\right),$$

where D is the diffusivity or mass transfer coefficient (m^2/h), A is the area through which the chemical is passing (m^2), C is the concentration of the diffusing chemical (g/m^3), and x is the distance being considered (m). The negative sign is simply the convention that the direction of diffusion is from high to low concentration (diffusion is positive when dC/dx is negative). Note that many scientists and texts define diffusion as an area specific process with units of g/m^2h and thus the area term (A) is not included in the diffusion equation. This is simply an alternative designation that describes transport as a flux density (g/m^2h) rather than as a flow (g/h). In either case the diffusion equation can be integrated numerically and even expressed in three dimensions using vector notation. However, for most environmental situations we usually have no accurate estimate of D or dx, so we combine the two into a one-dimensional mass transfer coefficient (k_M) with units of velocity (m/h). The chemical flux is then the product of this velocity, area, and concentration:

$$N = -k_M A C.$$

Mass transfer coefficients can be estimated from laboratory, mesocosm, and field studies and are widely used in environmental fate models. Mass transfer coefficients can be derived separately for molecular diffusion, turbulent diffusion, and dispersion in porous media, and all three terms can be added to the chemical flux equation. This is usually not necessary because one term often dominates the transport in specific environmental regions. Consider the vertical diffusion of methane gas generated by methanogenic bacteria in deep sediments. Molecular diffusion dominates in the highly compacted and low porosity deeper sediments. Dispersion becomes important as methane approaches the more porous surface sediments. Following methane gas ebulation from the sediment porewater, turbulent diffusion will dominate transport in a well-mixed water column (i.e., not a stagnant pool or beneath a thermocline where molecular diffusion will dominate). At the water surface, eddies tend to be damped and molecular diffusion may again dominate transport. Under stagnant atmospheric conditions (i.e., a temperature inversion) molecular diffusion will continue to dominate but will yield to more rapid mixing when typical turbulent conditions are reached. The magnitude and variability of the transport rate generally increase as the methane moves vertically through the environment, except when very stagnant conditions are encountered in the water or air. Modeling the transport of a chemical in air is particularly difficult because of the high spatial and temporal variability of air movement. Note also that advective processes in water or air usually transport chemicals at a faster rate than either molecular or turbulent diffusion.

Diffusion between Phases. The transport of a chemical between phases is sometimes treated as a third category of transport processes or even as a transformation reaction. Interphase or intermedia transport is not a transformation reaction because the chemical is moving only between phases; it is not reacting with anything or changing its chemical structure. Instead, intermedia transport is simply driven by diffusion between two phases. When a chemical reaches an interface such as air–water, particle–water, or (biological) membrane–water, two diffusive regions are created at either side of the interface. The classical description of this process is the Whitman two-film or two-resistance mass transfer theory, where chemicals pass through two stagnant boundary layers by molecular diffusion, while the two bulk phases are assumed to

be homogeneously mixed. This allows us to use a first-order function of the concentration gradient in the two phases, where the mass transfer coefficient will depend only on the molecular diffusivity of the chemical in each phase and the thickness of the boundary layers. This is fairly straightforward for transfer at the air–water interface (and often at the membrane–water interface), but not for the particle–water or particle–air interfaces.

Diffusive transport between phases can be described mathematically as the product of the departure from equilibrium and a kinetic term:

$$N = kA(C_1 - C_2K_{12}),$$

where N is the transport rate (g/h), k is a transport rate coefficient (m/h), A is the interfacial area (m^2), C_1 and C_2 are the concentrations in the two phases, and K_{12} is the equilibrium partition coefficient. At equilibrium K_{12} equals C_1/C_2, the term describing the departure from equilibrium ($C_1 - C_2K_{12}$) becomes zero, and thus the net rate of transfer also is zero. The partition coefficients are readily obtained from thermodynamic data and equilibrium partitioning experiments. The transport rate coefficients are usually estimated from the transport rate equation itself by measuring intermedia transport rates (N) under controlled laboratory conditions (temperature, wind and water velocities) at known values of A, C_1, C_2, and K_{12}. These measurements must then be extrapolated to the field, sometimes with great uncertainty. This uncertainty, along with the knowledge that many interfacial regions have reached or are near equilibrium, has led many to simply assume that equilibrium exists at the interface. Thus the net transport rate is zero and the phase distribution of a chemical is simply described by its equilibrium partition coefficient.

27.4 EQUILIBRIUM PARTITIONING

When a small amount of a chemical is added to two immiscible phases and then shaken, the phases will eventually separate and the chemical will partition between the two phases according to its solubility in each phase. The concentration ratio at equilibrium is the partition coefficient:

$$\frac{C_1}{C_2} = K_{12}.$$

In the laboratory, we usually determine K_{12} from the slope of C_1 versus C_2 over a range of concentrations. Partition coefficients can be measured for essentially any two-phase system: air–water, octanol–water, lipid–water, particle–water, and so on. In situ partition coefficients also can be measured where site-specific environmental conditions might influence the equilibrium phase distribution.

27.4.1 Air–Water Partitioning

Air–water partition coefficients ($K_{\text{air-water}}$) are essentially Henry's law constants (H):

$$K_{\text{air-water}} = H = \frac{C_{\text{air}}}{C_{\text{water}}},$$

where H can be expressed in dimensionless form (same units for air and water) or in units of pressure divided by concentration (e.g., $Pa\ m^3/mol$). The latter is usually written as

$$H = \frac{P_{air}}{C_{water}},$$

where P_{air} is the partial vapor pressure of the chemical. When H is not measured directly, it can be estimated from the ratio of the chemical's vapor pressure and aqueous solubility, although one must be careful about using vapor pressures and solubilities that apply to the same temperature and phase. Chemicals with high Henry's law constants (e.g., alkanes and many chlorinated solvents) have a tendency to escape from water to air and typically have high vapor pressures, low aqueous solubilities, and low boiling points. Chemicals with low Henry's law constants (e.g., alcohols, chlorinated phenols, larger polycyclic aromatic hydrocarbons, lindane, atrazine) tend to have high water solubility and/or very low vapor pressure. Note that some chemicals that are considered to be "nonvolatile," such as DDT, are often assumed to have low Henry's law constants. However, DDT also has a very low water solubility yielding a rather high Henry's law constant. Thus DDT readily partitions into the atmosphere as is now apparent from the global distribution of DDT.

27.4.2 Octanol–Water Partitioning

For many decades chemists have been measuring the octanol–water partition coefficient (K_{OW}) as a descriptor of hydrophobicity or how much a chemical "hates" to be in water. It is now one of the most important and frequently used physicochemical properties in toxicology and environmental chemistry. In fact toxicologists often simply use the symbol P, for partition coefficient, as if no other partition coefficient is important. Strong correlations exist between K_{OW} and many biochemical and toxicological properties. Octanol has a similar carbon:oxygen ratio as lipids, and the K_{OW} correlates particularly well with lipid–water partition coefficients. This has led many to use K_{OW} as a measure of lipophilicity or how much a chemical "loves" lipids. This is really not the case because most chemicals have an equal affinity for octanol and other lipids (within about a factor of ten), but their affinity for water varies by many orders of magnitude. Thus it is largely aqueous solubility which determines K_{OW} not octanol or lipid solubility. We generally express K_{OW} as log K_{OW} because K_{OW} values range from less than one (alcohols) to over one billion (larger alkanes and alkyl benzenes).

27.4.3 Lipid–Water Partitioning

In most cases we can assume that the equilibrium distribution and partitioning of organic chemicals in both mammalian and nonmammalian systems is a function of lipid content in the animal and that the lipid–water partition coefficient (K_{LW}) is equal to K_{OW}. Instances where this is not the case include specific binding sites (e.g., kepone in the liver) and nonequilibrium conditions caused by slow elimination rates of higher level organisms or structured lipid phases that sterically hinder accumulation of very hydrophobic chemicals. For aquatic organisms in constant contact with water, the bioconcentration factor or fish-water partition coefficient (K_{FW}) is simply:

$$K_{FW} = f_{lipid} K_{OW},$$

where f_{lipid} is the mass fraction of lipid in the fish (g lipid/g fish). Several studies have shown this relationship works well for many fish and shellfish species and an aggregate plot of K_{FW} versus K_{OW} for many different fish species yields a slope of 0.048, which is about the average lipid concentration of fish (5%). Again, nonequilibrium conditions will cause deviation from this equation. Such deviations are found at both the top and bottom of the aquatic food chain. Phytoplankton can have higher apparent lipid–water partition coefficients because their large surface area : volume ratios increase the relative importance of surface sorption. Top predators such as marine mammals also have high apparent lipid-water partition coefficients because of very slow elimination rates. Thus the deviations occur not because "there is something wrong with the equation" but because the underlying assumption of equilibrium is not appropriate in these cases.

27.4.4 Particle–Water Partitioning

It has been known for several decades that many chemicals preferentially associate with soil and sediment particles rather than the aqueous phase. The particle–water partition coefficient (K_{P}) describing this phenomenon is

$$K_{\text{P}} = \frac{C_{\text{S}}}{C_{\text{W}}},$$

where C_{S} is the concentration of chemical in the soil or sediment (mg/kg dry weight) and C_{W} is the concentration in water (mg/L). In this form, K_{P} has units of L/kg or reciprocal density. Dimensionless partition coefficients are sometimes used where K_{P} is multiplied by the particle density (in kg/L). It has also been observed, first by pesticide chemists in soil systems and later by environmental engineers and chemists in sewage effluent and sediment systems, that nonionic organic chemicals were primarily associated with the organic carbon phase(s) of particles. A plot of K_{P} versus the mass fraction of organic carbon in the soil (f_{OC}, g/g) is linear with a near-zero intercept yielding the simple relationship

$$K_{\text{P}} = f_{\text{OC}} K_{\text{OC}},$$

where K_{OC} is the organic carbon–water partition coefficient (L/kg). Studies with many chemicals and many sediment/soil systems have demonstrated the utility of this equation when the fraction of organic carbon is about 0.5% or greater. At lower organic carbon fractions, interaction with the mineral phase becomes relatively more important (though highly variable) resulting in a small positive intercept of K_{P} versus f_{OC}. The strongest interaction between organic chemicals and mineral phases appears to be with dry clays. Thus K_{P} will likely change substantially as a function of water content in low organic carbon, clay soils.

Measurements of K_{OC} have been taken directly from partitioning experiments in sediment–and soil–water systems over a range of environmental conditions in both the laboratory and the field. Not surprisingly, the K_{OC} values for many organic chemicals are highly correlated with their K_{OW} values. Plots of the two partition coefficients for hundreds of chemicals with widely ranging K_{OW} values yield slopes from about 0.3 to 1, depending on the classes of compounds and the particular methods included. Most fate modelers continue to use a slope of 0.41, which was reported by the first definitive

study on the subject in the early 1980s. Thus we now have a means of estimating the partitioning of a chemical between a particle and water by using the K_{OW} and f_{OC}:

$$K_P = f_{OC} K_{OC} = f_{OC}\ 0.41 K_{OW}.$$

This relationship is commonly used in environmental fate models to predict aqueous concentrations from sediment measurements by substituting the equilibrium expression for K_P and rearranging to solve for C_W:

$$K_P = \frac{C_S}{C_W} = f_{OC}\ 0.41 K_{OW},$$

$$C_W = \frac{C_S}{f_{OC} 0.41 K_{OW}}.$$

This last equation forms the basis for the EPA's sediment quality guidelines that are used to assess the potential toxicity of contaminated sediments. The idea is to simply measure C_S and f_{OC}, look up K_{OW} in a table, compute the predicted C_W, and compare this result to established water quality criteria for the protection of aquatic life or human life (e.g., carcinogenicity risk factors). The use of this simple equilibrium partitioning expression for this purpose is currently the subject of much debate among scientists as well as policy makers.

27.5 TRANSFORMATION PROCESSES

The potential environmental hazard associated with the use of a chemical is directly related to it's persistence in the environment (see Chapter 26), which in turn depends on the rates of chemical transformation reactions. Transformation reactions can be divided into two classes: reversible reactions that involve continuous exchange among chemical states (ionization, complexation) and irreversible reactions that permanently transform a parent chemical into a daughter or reaction product (photolysis, hydrolysis, and many redox reactions). Reversible reactions are usually abiotic, although biological processes can still exert great influence over them (e.g., via production of complexing agents or a change in pH). Irreversible reactions can be abiotic or mediated directly by biota, particularly bacteria.

27.5.1 Reversible Reactions

Ionization. Ionization refers to the dissociation of a neutral chemical into charged species. The most common form of neutral toxicant dissociation is acid-base equilibria. The hypothetical monoprotic acid, HA, will dissociate in water to form the conjugate acid-base pair (H^+, A^-) usually written as

$$HA + H_2O = H_3O^+ + A^-.$$

The equilibrium constant for this reaction, the acidity constant (K_a), is defined by the law of mass action and is given by

$$K_a = \frac{[H_3O^+][A^-]}{[HA]}.$$

For convenience we often express equilibrium constants as the negative logarithm, or pK value. Thus the relative proportion of the neutral and charged species, will be a function of the pKa and solution pH. When the pH is equal to pKa, equal concentrations of the neutral and ionized forms will be present. When pH is less than the pKa, the neutral species will be predominant; when pH is greater than pKa, the ionized species will be in excess. The exact equilibrium distribution can be calculated from the equilibrium expression above and the law of mass conservation.

The fate of a chemical is often a function of the relative abundance of a particular chemical species as well as the total concentration. For example, the neutral chemical might partition into biological lipids or organic carbon in soil to a greater extent than the ionized form. Many acidic toxicants (pentachlorophenol) exhibit higher toxicities to aquatic organisms at lower pH where the neutral species predominates. However, specific ionic interactions will take place only with the ionized species. A classic example of how pH influences the fate and effects of a toxicant is with hydrogen cyanide. The pKa of HCN is about 9 and the toxicity of CN^- is much higher than that of HCN for many aquatic organisms. Thus the discharge of a basic (high pH) industrial effluent containing cyanide would pose a greater hazard to fish than a lower pH effluent (everything else being equal). The effluent could be treated to reduce the pH well below the pKa according to the reaction:

$$CN^- + H^+ = HCN_{(aq)},$$

thus reducing the hazard to the fish. However, HCN has a rather high Henry's law constant and will partition into the atmosphere:

$$HCN_{(aq)} = HCN_{(air)}.$$

This may be fine for the fish, but birds in the area and humans working at the industrial plant will now have a much greater exposure to HCN. Thus both the fate and toxicity of a chemical can be influenced by simple ionization reactions.

Precipitation and Dissolution. A special case of ionization is the dissolution of a neutral solid phase into soluble species. For example, the binary solid metal sulfide, CuS, dissolves in water according to

$$CuS(s) + H^+ = Cu^{2+} + HS^-.$$

The equilibrium constant for this reaction, the solubility product (K_{sp}), is given by

$$K_{sp} = \frac{[Cu^{2+}][HS^-]}{[H^+]}.$$

The solubility product for CuS is very low ($K_{sp} = 10^{-19}$ as written) so that the presence of sulfide in water acts to immobilize Cu (and many other metals) and reduce effective exposure. The formation of metal sulfides is important in anaerobic soil and sediment, stagnant ponds and basins, and many industrial and domestic sewage treatment plants and discharges. Co-precipitation of metals also can be a very important removal process in natural waters. In aerobic systems, the precipitation of hydrous

oxides of manganese and iron often incorporate other metals as impurities. In anaerobic systems, the precipitation of iron sulfides can include other metals as well. These co-precipitates are usually not thermodynamically stable, but their conversion to stable mineral phases often takes place on geological time scales.

Complexation and Chemical Speciation. Natural systems contain many chemicals that undergo ionic or covalent interactions with toxicants to change toxicant speciation and chemical speciation can have a profound effect on both fate and toxicity. Again, in the case of copper, inorganic ions (Cl^-, OH^-) and organic detritus (humic acids, peptides) will react with dissolved Cu^{2+} to form various metal-ligand complexes. Molecular diffusivities of complexed copper will be lower than uncomplexed (hydrated) copper and will generally decrease with the size and number of ligands. The toxicity of free, uncomplexed Cu^{2+} to many aquatic organisms is much higher than Cu^{2+} that is complexed to chelating agents such as EDTA or glutathione. Many transition metal toxicants, such as Cu, Pb, Cd, and Hg, have high binding constants with compounds that contain amine, sulfhydryl, and carboxylic acid groups. These groups are quite common in natural organic matter. Even inorganic complexes of OH^- and Cl^- reduce Cu^{2+} toxicity. In systems where a mineral phase is controlling Cu^{2+} solubility, the addition of these complexing agents will shift the solubility equilibrium according to LeChatelier's principle as shown here for CuS and OH^-, Cl^-, and GSH (glutathione):

$$\begin{aligned}
&CuS(s) + H^+ = Cu^{2+} + HS^-, && K_{sp},\\
&Cu^{2+} + OH^- = CuOH^+, && K_{CuOH},\\
&Cu^{2+} + Cl^- = CuCl^+, && K_{CuCl},\\
&Cu^{2+} + GSH = CuGS^+ + H^+, && K_{CuGS}.
\end{aligned}$$

Each successive complexation reaction "leaches" Cu^{2+} from the solid mineral phase, thereby increasing the total copper in the water but not affecting the concentration of (or exposure to) Cu^{2+}. These equilibria can be combined into one reaction:

$$4CuS(s) + 3H^+ + OH^- + Cl^- + GSH = Cu^{2+} + 4HS^- + CuOH^+ + CuCl^+ + CuGS^+,$$

and the overall equilibrium constant derived as shown:

$$\begin{aligned}
K_{overall} &= (4)K_{sp} \times K_{CuOH} \times K_{CuCl} \times K_{CuGS}\\
&= [Cu^{2+}]\,[CuOH^+]\,[CuCl^+]\,[CuGS^+]\,[HS^-]^4/[H^+]^3\,[OH^-]\,[Cl^-]\,[GS^-].
\end{aligned}$$

A series of simultaneous equations can be derived for these reactions to compute the concentration of individual copper species, and the total concentration of copper, $[Cu]_T$, would be given by

$$[Cu]_T = [Cu^{2+}] + [CuOH^+] + [CuCl^+] + [CuGS^+].$$

Thus the total copper added to a toxicity test or measured as the exposure (e.g., by atomic absorption spectroscopy) may be much greater than that which is available to an organism to induce toxicological effects.

Literally hundreds of complex equilibria like this can be combined to model what happens to metals in aqueous systems. Numerous speciation models exist for this application that include all of the necessary equilibrium constants. Several of these models include surface complexation reactions that take place at the particle–water interface. Unlike the partitioning of hydrophobic organic contaminants into organic carbon, metals actually form ionic and covalent bonds with surface ligands such as sulfhydryl groups on metal sulfides and oxide groups on the hydrous oxides of manganese and iron. Metals also can be biotransformed to more toxic species (e.g., conversion of elemental mercury to methyl-mercury by anaerobic bacteria), less toxic species (oxidation of tributyl tin to elemental tin), or temporarily immobilized (e.g., via microbial reduction of sulfate to sulfide, which then precipitates as an insoluble metal sulfide mineral).

27.5.2 Irreversible Reactions

The reversible transformation reactions discussed above alter the fate and toxicity of chemicals, but they do not irreversibly change the structure or properties of the chemical. An acid can be neutralized to its conjugate base, and vice versa. Copper can precipitate as a metal sulfide, dissolve and from a complex with numerous ligands, and later re-precipitate as a metal sulfide. Irreversible transformation reactions alter the structure and properties of a chemical forever.

Hydrolysis. Hydrolysis is the cleavage of organic molecules by reaction with water with a net displacement of a leaving group (X) with OH^-:

$$RX + H_2O = ROH + HX.$$

Hydrolysis is part of the larger class of chemical reactions called nucleophilic displacement reactions in which a nucleophile (electron-rich species with an unshared pair of electrons) attacks an electrophile (electron deficient), cleaving one covalent bond to form a new one. Hydrolysis is usually associated with surface waters but also takes place in the atmosphere (fogs and clouds), groundwater, at the particle–water interface of soils and sediments, and in living organisms.

Hydrolysis can proceed through numerous mechanisms via attack by H_2O (neutral hydrolysis) or by acid (H^+) or base (OH^-) catalysis. Acid and base catalyzed reactions proceed via alternative mechanisms that require less energy than neutral hydrolysis. The combined hydrolysis rate term is a sum of these three constituent reactions and is given by

$$\frac{d[RX]}{dt} = k_{obs}[RX] = k_a[H^+][RX] + k_n[RX] + k_b[OH^-][RX],$$

where [RX] is the concentration of the hydrolyzable chemical, k_{obs} is the macroscopic observed hydrolysis rate constant, and k_a, k_n, and k_b are the rate constants for the acid-catalyzed, neutral, and base-catalyzed hydrolysis. If we assume that the hydrolysis can be approximated by first-order kinetics with respect to RX (which is usually true), the rate term is reduced to

$$k_{obs} = k_a[H^+] + k_n + k_b[OH^-].$$

Neutral hydrolysis is dependent only on water which is present in excess, so k_n is a simple pseudo-first-order rate constant (with units t^{-1}). The acid- and base-catalyzed

hydrolysis depend on the molar quantities of $[H^+]$ and $[OH^-]$, respectively, so k_a and k_b have units of $M^{-1}t^{-1}$.

The observed or apparent hydrolysis half-life at a fixed pH is then given by

$$t_{1/2} = \frac{\ln 2}{k_{obs}}.$$

Compilations of hydrolysis half-lives at pH and temperature ranges encountered in nature can be found in many sources. Reported hydrolysis half-lives for organic compounds at pH 7 and 298 K range at least 13 orders of magnitude. Many esters hydrolyze within hours or days, whereas some organic chemicals will never hydrolyze. For halogenated methanes, which are common groundwater contaminants, half-lives range from about 1 year for CH_3Cl to about 7000 years for CCl_4. The half-lives of halomethanes follows the strength of the carbon-halogen bond with half-lives decreasing in the order F > Cl > Br. Small structural changes can dramatically alter hydrolysis rates. An example is the difference between tetrachloroethane ($Cl_2HC–CHCl_2$) and tetrachloroethene ($Cl_2C=CCl_2$) which have hydrolysis half-lives of about 0.5 year and 10^9 years, respectively. In this case the hydrolysis rate is affected by the C–Cl bond strength and the steric bulk at the site of nucleophilic substitution.

The apparent rate of hydrolysis and the relative abundance of reaction products is often a function of pH because alternative reaction pathways are preferred at different pH. In the case of halogenated hydrocarbons, base-catalyzed hydrolysis will result in elimination reactions while neutral hydrolysis will take place via nucleophilic displacement reactions. An example of the pH dependence of hydrolysis is illustrated by the base-catalyzed hydrolysis of the structurally similar insecticides DDT and methoxychlor. Under a common range of natural pH (5 to 8) the hydrolysis rate of methoxychlor is invariant while the hydrolysis of DDT is about 15-fold faster at pH 8 compared to pH 5. Only at higher pH (>8) does the hydrolysis rate of methoxychlor increase. In addition the major product of DDT hydrolysis throughout this pH range is the same (DDE), while the methoxychlor hydrolysis product shifts from the alcohol at pH 5–8 (nucleophilic substitution) to the dehydrochlorinated DMDE at pH > 8 (elimination). This illustrates the necessity to conduct detailed mechanistic experiments as a function of pH for hydrolytic reactions.

Photolysis. Photolysis of a chemical can proceed either by direct absorption of light (direct photolysis) or by reaction with another chemical species that has been produced or excited by light (indirect photolysis). In either case photochemical transformations such as bond cleavage, isomerization, intramolecular rearrangement, and various intermolecular reactions can result. Photolysis can take place wherever sufficient light energy exists, including the atmosphere (in the gas phase and in aerosols and fog/cloud droplets), surface waters (in the dissolved phase or at the particle–water interface), and in the terrestrial environment (on plant and soil/mineral surfaces).

Photolysis dominates the fate of many chemicals in the atmosphere because of the high solar irradiance. Near the earth's surface, chromophores such as nitrogen oxides, carbonyls, and aromatic hydrocarbons play a large role in contaminant fate in urban areas. In the stratosphere, light is absorbed by ozone, oxygen, organohalogens, and hydrocarbons with global environmental implications. The rate of photolysis in surface waters depends on light intensity at the air–water interface, the transmittance through

this interface, and the attenuation through the water column. Open ocean waters ("blue water") can transmit blue light to depths of 150 m while highly eutrophic or turbid waters might absorb all light within 1 cm of the surface.

Oxidation-Reduction Reactions. Although many redox reactions are reversible, they are included here because many of the redox reactions that influence the fate of toxicants are irreversible on the temporal and spatial scales that are important to toxicity.

Oxidation is simply defined as a loss of electrons. Oxidizing agents are electrophiles and thus gain electrons upon reaction. Oxidations can result in the increase in the oxidation state of the chemical as in the oxidation of metals or oxidation can incorporate oxygen into the molecule. Typical organic chemical oxidative reactions include dealkylation, epoxidation, aromatic ring cleavage, and hydroxylation. The term autooxidation, or weathering, is commonly used to describe the general oxidative degradation of a chemical (or chemical mixture, e.g., petroleum) upon exposure to air. Chemicals can react abiotically in both water and air with oxygen, ozone, peroxides, free radicals, and singlet oxygen. The last two are common intermediate reactants in indirect photolysis. Mineral surfaces are known to catalyze many oxidative reactions. Clays and oxides of silicon, aluminum, iron, and manganese can provide surface active sites that increase rates of oxidation. There are a variety of complex mechanisms associated with this catalysis, so it is difficult to predict the catalytic activity of soils and sediment in nature.

Reduction of a chemical species takes place when an electron donor (reductant) transfers electrons to an electron acceptor (oxidant). Organic chemicals typically act as the oxidant, while abiotic reductants include sulfide minerals, reduced metals or sulfur compounds, and natural organic matter. There are also extracellular biochemical reducing agents such as porphyrins, corrinoids, and metal-containing coenzymes. Most of these reducing agents are present only in anaerobic environments where anaerobic bacteria are themselves busy reducing chemicals. Thus it is usually very difficult to distinguish biotic and abiotic reductive processes in nature. Well-controlled, sterile laboratory studies are required to measure abiotic rates of reduction. These studies indicate that many abiotic reductive transformations could be important in the environment, including dehalogenation, dealkylation, and the reduction of quinones, nitrosamines, azoaromatics, nitroaromatics, and sulfoxides. Functional groups that are resistant to reduction include aldehydes, ketones, carboxylic acids (and esters), amides, alkenes, and aromatic hydrocarbons.

Biotransformations. As we have seen throughout much of this textbook, vertebrates have developed the capacity to transform many toxicants into other chemicals, sometimes detoxifying the chemical and sometimes activating it. The same or similar biochemical processes that hydrolyze, oxidize, and reduce toxicants in vertebrates also take place in many lower organisms. In particular, bacteria, protozoans, and fungi provide a significant capacity to biotransform toxicants in the environment. Although many vertebrates can metabolize toxicants faster than these lower forms of life, the aggregate capacity of vertebrates to biotransform toxicants (based on total biomass and exposure) is insignificant to the overall fate of a toxicant in the environment. In this section we use the term *biotransformation* to include all forms of direct biological transformation reactions.

Biotransformations follow a complex series of chemical reactions that are enzymatically mediated and are usually irreversible reactions that are energetically favorable, resulting in a decrease in the Gibbs free energy of the system. Thus the potential for biotransformation of a chemical depends on the reduction in free energy that results from reacting the chemical with other chemicals in its environment (e.g., oxygen). As with inorganic catalysts, microbes simply use enzymes to lower the activation energy of the reaction and increase the rate of the transformation. Each successive chemical reaction further degrades the chemical, eventually mineralizing it to inorganic compounds (CO_2, H_2O, inorganic salts) and continuing the carbon and hydrologic cycles on earth.

Usually microbial growth is stimulated because the microbes capture the energy released from the biotransformation reaction. As the microbial population expands, overall biotransformation rates increase, even though the rate for each individual microbe may be constant or even decrease. This complicates the modeling and prediction of biotransformation rates in nature. When the toxicant concentrations (and potential energy) are small relative to other substrates or when the microbes cannot efficiently capture the energy from the biotransformation, microbial growth is not stimulated but biotransformation often still proceeds inadvertently through cometabolism.

Biotransformation can be modeled using simple Michaelis-Menten enzyme kinetics, Monod microbial growth kinetics, or more complex numerical models that incorporate various environmental parameters, and even the formation of microbial mats or slime, which affects diffusion of the chemical and nutrients to the microbial population. Microbial ecology involves a complex web of interaction among numerous environmental processes and parameters. The viability of microbial populations and the rates of biotransformation depend on many factors such as genetic adaptation, moisture, nutrients, oxygen, pH, and temperature. Although a single factor may limit biotransformation rates at a particular time and location, we cannot generalize about what limits biotransformation rates in the environment. Biotransformation rates often increase with temperature (according to the Arrhenius law) within the optimum range that supports the microbes, but many exceptions exist for certain organisms and chemicals. The availability of oxygen and various nutrients (C, N, P, Fe, Si) often limits microbial growth, but the limiting nutrient often changes with space (e.g., downriver) and time (seasonally and even diurnally).

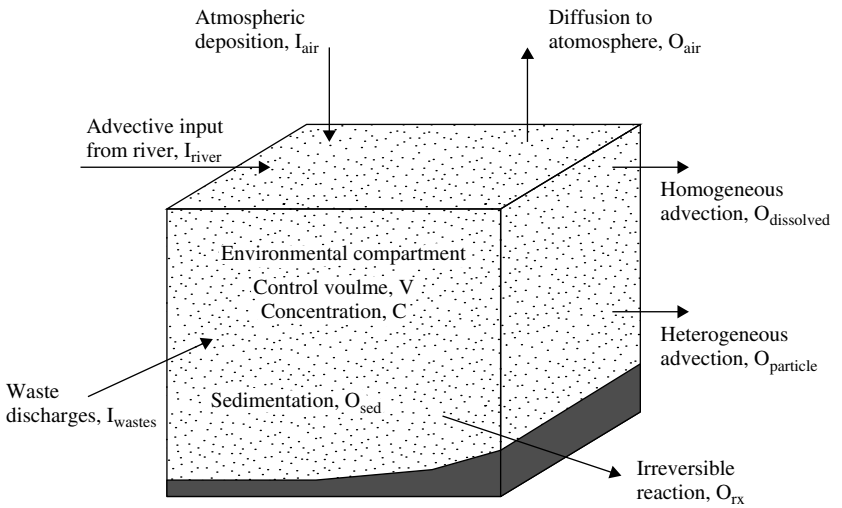
Long-term exposure of microbial populations to certain toxicants often is necessary for adaptation of enzymatic systems capable of degrading those toxicants. This was the case with the Exxon Valdez oil spill in Alaska in 1989. Natural microbial populations in Prince William Sound, Alaska, had developed enzyme systems that oxidize petroleum hydrocarbons because of long-term exposure to natural oil seeps and to hydrocarbons that leached from the pine forests in the area. Growth of these natural microbial populations was nutrient limited during the summer. Thus the application of nutrient formulations to the rocky beaches of Prince William Sound stimulated microbial growth and helped to degrade the spilled oil.

In terrestrial systems with high nutrient and oxygen content, low moisture and high organic carbon can control biotransformation by limiting microbial growth and the availability of the toxicant to the microbes. For example, biotransformation rates of certain pesticides have been shown to vary two orders of magnitude in two separate agricultural fields that were both well aerated and nutrient rich, but spanned the common range of moisture and organic carbon content.

27.6 ENVIRONMENTAL FATE MODELS

The discussion above provides a brief qualitative introduction to the transport and fate of chemicals in the environment. The goal of most fate chemists and engineers is to translate this qualitative picture into a conceptual model and ultimately into a quantitative description that can be used to predict or reconstruct the fate of a chemical in the environment (Figure 27.1). This quantitative description usually takes the form of a mass balance model. The idea is to compartmentalize the environment into defined units (control volumes) and to write a mathematical expression for the mass balance within the compartment. As with pharmacokinetic models, transfer between compartments can be included as the complexity of the model increases. There is a great deal of subjectivity to assembling a mass balance model. However, each decision to include or exclude a process or compartment is based on one or more assumptions—most of which can be tested at some level. Over time the applicability of various assumptions for particular chemicals and environmental conditions become known and model standardization becomes possible.

The construction of a mass balance model follows the general outline of this chapter. First, one defines the spatial and temporal scales to be considered and establishes the environmental compartments or control volumes. Second, the source emissions are identified and quantified. Third, the mathematical expressions for advective and diffusive transport processes are written. And last, chemical transformation processes are quantified. This model-building process is illustrated in Figure 27.4. In this example we simply equate the change in chemical inventory (total mass in the system) with the difference between chemical inputs and outputs to the system. The inputs could include numerous point and nonpoint sources or could be a single estimate of total chemical load to the system. The outputs include all of the loss mechanisms: transport



Inventory change = inputs (I) – outputs (O)

$$Vdc/dt = (I_{air} + I_{river} + I_{wastes}) - (O_{air} + O_{sed} + O_{dissolved} + O_{particle} + O_{rx})$$

at steady state, $dc/dt = 0$ and $(I_{air} + I_{river} + I_{wastes}) = (O_{air} + O_{sed} + O_{dissolved} + O_{particle} + O_{rx})$

Figure 27.4 A simple chemical mass balance model.

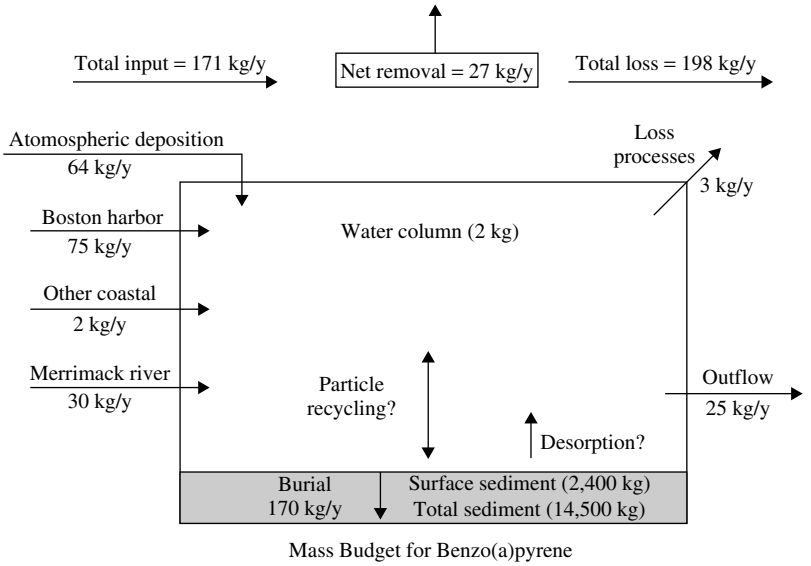


Figure 27.5 Information provided by a chemical mass balance model. The annual mass budget of benzo[*a*]pyrene in Massachusetts Bay is shown.

out of the compartment and irreversible transformation reactions. If steady state can be assumed (i.e., the chemical's concentration in the compartment is not changing over the time scale of the model), the inventory change is zero and we are left with a simple mass balance equation to solve. Unsteady-state conditions would require a numerical solution to the differential equations.

There are many tricks and shortcuts to this process. For example, rather than compiling all of the transformation rate equations (or conducting the actual kinetic experiments yourself), there are many sources of typical chemical half-lives based on pseudo-first-order rate expressions. It is usually prudent to begin with these "best estimates" of half-lives in air, water, soil, and sediment and perform a sensitivity analysis with the model to determine which processes are most important. One can return to the most important processes to assess whether more detailed rate expressions are necessary. An illustration of this mass balance approach is given in Figure 27.5 for benzo[*a*]pyrene. This approach allows a first-order evaluation of how chemicals enter the environment, what happens to them in the environment, and what the exposure concentrations will be in various environmental media. Thus the chemical mass balance provides information relevant to toxicant exposure to both humans and wildlife.

SUGGESTED READING

Hemond, H. F., and E. J. Fechner. *Chemical Fate and Transport in the Environment*. New York: Academic Press, 1994.

Mackay, D., W. Y. Shiu, and K. C. Ma. *Physical-Chemical Properties and Environmental Fate and Degradation Handbook*. CRCnetBASE 2000 CR-ROM. Boca Raton, FL: CRC Press, 2000.

Mackay, D. *Multimedia Environmental Models: The Fugacity Approach*, 2nd ed. Boca Raton, FL: Lewis Publishers, 2001.

Rand, G. M., ed. *Fundamentals of Aquatic Toxicology: Part II Environmental Fate*. Washington, DC: Taylor and Francis, 1995.

Schnoor, J. L. *Environmental Modeling: Fate and Transport of Pollutants in Water, Air, and Soil*. New York: Wiley, 1996.

Schwarzenbach, R. P., P. M. Gschwend, and D. M. Imboden. *Environmental Organic Chemistry*, 2nd ed. New York: Wiley, 2002.