PART VII

ENVIRONMENTAL TOXICOLOGY

CHAPTER 24

Toxicant Analysis: Analytical Methods and Quality Assurance

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24.1 INTRODUCTION

Some estimates suggest that there are some 200,000 chemicals synthesized annually worldwide. At the most fundamental level, the risk of one of these compounds causing harm to a living organism is a function of two things: the toxicity of that compound to the species of interest and the organism's exposure to the compound. The former has largely been the subject of this text so far. This chapter will begin to examine the latter.

Today's analytical chemist has access to analytical tools for the detection and quantification of a wide variety of potentially toxic compounds, at concentrations that are increasingly miniscule. Analytical laboratories routinely report concentrations of chemicals in the subparts-per-billion range. To try and envision this infinitesimally small number, consider the following analogy. A part per million is akin to a single white ping-pong ball in a railroad hopper car full of one million colored ping-pong balls. A part per billion is akin to a single white ping-pong ball in 1000 railroad hopper cars (i.e., a train stretching for ~9.5 mi) full of colored ping-pong balls. Although these advances have tremendous benefits, this can be a double-edged sword. Many chemicals can now be detected at concentrations far below any level of toxicological significance, whereas there is a public perception that if a chemical can be detected (e.g., in human serum), then it must be causing deleterious effects.

Although new techniques and instruments continue to enter the commercial market, the basic analytical process has not changed:

- Define the research goal(s).
- Identify appropriate techniques and methods.
- Develop a sampling scheme to obtain representative samples.
- Isolate the compound(s) of interest.
- Remove potential interfering components.
- Quantify and evaluate the data in relation to the original research goals.

A Textbook of Modern Toxicology, *Fourth Edition*. Edited by Ernest Hodgson

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. This chapter is concerned with the sampling, isolation, separation, and measurement of toxicants, including the various quality assurance (QA) and quality control (QC) measures employed to assure the accuracy and precision of the data.

24.2 ENVIRONMENTAL SAMPLE COLLECTION METHODS

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. A great deal of the error in measurements, such as the ones discussed in this chapter, is introduced in the sample collection preservation and storage. Therefore, a great deal of effort should go into the planning of this phase of the study, and care must be taken to ensure that the resulting data meet the objectives of the study. Often, special attention to sampling procedures is necessary. Ultimately, the initial hypothesis and goals of the study will determine what the most appropriate sampling schemes and analytical methods will be.

24.2.1 Sampling Schemes

The goal in developing a sampling scheme is to come up with a sample that truly reflects the composition of the matrix to be analyzed within the context of the study aims; in other words, a *representative sample*. This can be challenging as contamination is rarely, if ever, uniformly distributed. This is particularly true of solid matrices. Finally, not only can contamination vary spatially but temporally as well. Sampling schemes generally fall into three categories: *random*, *systematic*, and *judgmental* (Figure 24.1).

Random Sampling From a statistical standpoint, a random sampling design is really the only choice. In a random design, sampling locations within the area of interest (AOI) are determined using a method that prevents any bias in the selection. For example, one could use a random number generator to generate latitude/longitude coordinates within the confines of the area. This type of design is useful if one is trying to determine baseline conditions at a reference site. In this case, a robust random sampling design, with a sufficient number of samples, will have a high degree of statistical power, giving the investigator confidence to draw conclusions about the overall site conditions.

There are, however, a number of challenges that may limit the utility of this type of design. First, to achieve a high degree of statistical power, a large number of samples are often required. Given that collection and analysis of environmental samples is often quite expensive, the number of Samples required can make it cost prohibitive. Second, in a very heterogeneous AOI, random sampling locations can easily fall in locations that are not able to be sampled or would not make sense to sample (e.g., in the middle of a street or building). Finally, the nature of this type of

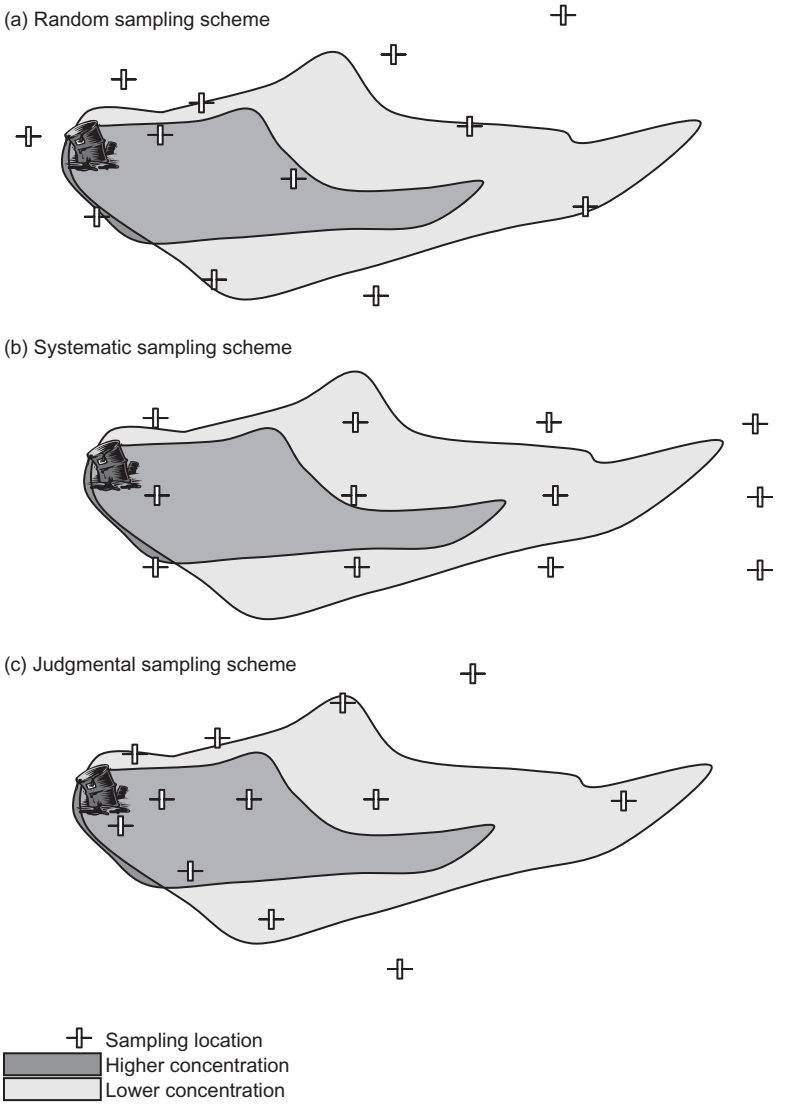

Figure 24.1 Sampling schemes: (a) random, (b) systematic, and (c) judgmental sampling designs.

design can lead to "holes" in the sampling scheme with other areas having large numbers of samples. This last problem can be alleviated with the use of a systematic sampling scheme.

Systematic Sampling In this type of sampling scheme, the AOI is blocked off with a grid and a sample is taken from within each of the grid squares. This type of scheme is useful if one wants to be assured of consistent sample coverage of the AOI. The disadvantage here is that one loses most (if not all) of that ability to perform statistical analyses of the data.

Judgmental Sampling Often, with environmental contamination, the source of the contamination is known. What needs to be determined is the nature and extent of the contamination (e.g., what are the contaminants and how far have they traveled from the source). Therefore, this is one of the most common sampling schemes used in environmental toxicological and environmental exposure assessment settings. The sampling scheme is designed such that it maximizes the number of samples taken in and around the source of contamination. Sampling locations can then move out from the source along transects, with increasing distance between samples as one moves further from the source. The disadvantage with a judgmental sampling design is that there may be secondary sources of contamination that are not known and could thus be missed.

24.2.2 Environmental Matrices

The most common environmental matrices include water, air, solid (soil, sediment, or sludge), biota, and vegetation. With the exception of air samples, each of these sample types consists of a complex matrix that can include many and varied constituents in addition to the analytes of interest. Each of these sample types requires unique strategies to collect a representative sample.

Water One of the most common types of environmental matrices is water. Many factors must be considered to obtain representative samples of water. The most important factor is the contaminant of potential concern (COPC) and the point at which it entered the aquatic environment. Pollutants can be contributed by agricultural, industrial, municipal, or other sources, such as spills. Other factors include the velocity of stream or river flow (or velocity and direction in the case of groundwater), temperature, thermal and salinity stratification, and sediment content.

The simplest method of collecting water is the "grab" technique, whereby a container is lowered into the water, rinsed, filled, and capped. Specialized samplers can be used to obtain water at greater depths. In the case of groundwater, samples are obtained using a groundwater well or a piezometer, the latter being easier and cheaper to install. The collection of groundwater samples is far more involved and expensive than collecting a surface water sample; therefore, much more effort is put into determining locations for sampling. Obtaining a representative sample is affected not only by where but also when to collect a sample. Sources of contamination that are intermittent (e.g., pesticide runoff) will vary greatly by the time of year that a sample is taken. For this reason, the use of passive sampling devices (PSDs) is increasingly common. PSDs come in a variety of configurations, but the general design principles are the same: a clean adsorptive phase is placed in contact with the media of interest (water) for a period of time (generally, about 1 month). Contaminants in the water that have an affinity for the adsorbent will partition into the PSD at a constant rate known as the sampling rate. Using the known sampling rate, one can calculate the average water concentration over the time period that the PSD was deployed. In this way, the PSD acts as an "integrative sampler." The PSD has the added advantage that it acts to concentrate ultra-trace levels of chemicals present in the water, making them detectable with conventional laboratory methods. Finally, PSDs deployed in water will only sequester contaminants that are

freely dissolved; therefore, they can provide an estimation of the bioavailability of contaminants.

Air Most pollutants entering the atmosphere come from fuel combustion, industrial processes, and solid waste disposal. Additional miscellaneous sources, such as 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 (see Chapter 18). 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.

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/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 0.01–10.0 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 10–50 L of air per minute, and insecticides can be collected in smaller "midget" impingers that handle flows of 2.0–4.5 L of air per minute. The solvent within the impinger will vary depending on the COPCs. 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. Finally, PSDs have also been used to sample airborne contaminants.

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 the 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 cm and 7–15 cm) 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 (~25 cm) that incorporates a blade to slice a core of soil after placing the sampler at the desired depth.

Biota The collection of biota for the purpose of environmental monitoring is primarily done in the aquatic environment, although terrestrial and avian species may be sampled as well. Sampling techniques fall into three general categories. First is the collection of nonlethal samples, for example, hair or feathers for mercury analysis. The second category is the collection of feral organisms such as fish or mussels. An example of this is the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends Mussel Watch program. In this program, mussel and sediment samples have been collected for analysis of over 100 organic and inorganic constituents, from 300 coastal sites around the United States. This program has been operating since 1986. This type of sampling has the advantage of giving a direct measure of the actual body burden of contaminants for organisms in the AOI. The final category is the use of bioassays, where caged "clean" organisms (fish or mussels) are placed in the sampling area for a period of time to allow them to accumulate site-related contaminants. This type of assay has the advantage of reducing the potential spatial variability errors introduced from trapping feral organisms such as fish. The disadvantage here is that caged organisms occasionally suffer from low survivorship and/or succumb to predation.

24.3 ANALYTICAL TECHNIQUES

Once the environmental samples have been collected, they have to be prepared for analysis. First, the analytes of interest have to be extracted from the bulk medium (water, soil, etc.). During this process, many analytically interfering compounds will also be extracted from the bulk medium. Therefore, some type of cleaning and concentration step has to be performed. Finally, the clean and concentrated sample extract is analyzed using an appropriate technique/instrument, and the mass of the chemicals present in the original sample can be estimated.

24.3.1 Extraction Techniques

In most cases, the analysis of the COPC 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 5–25 vol of solvent to 1 vol of sample. One or more of five 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 5–15 min 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 quantification 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. Large wattage (e.g., 450 W) sonicators are used to extract compounds from environmental samples, and several United States Environmental Protection Agency (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.

Solid-Phase Extraction (SPE) In SPE, water samples are filtered through a cartridge or filter disk made of material such as C-18. Analytes of interest are retained on the SPE filter and can be collected by eluting with different solvents or solvent mixtures. This general process will be discussed further in the next section.

Continuous Extraction The procedure, called Soxhlet extraction, is performed on solid samples (e.g., soil) and involves the use of an organic solvent or a combination of solvents. The sample is weighed into a cup (thimble) of specialized porous material such as cellulose or fiberglass and is 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 the boiling point, 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 the sample matrix, the extraction can be completed in as little as 2 h but may

take as long as 3–4 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 with 250 mL). In addition, specialized glassware can be used to perform continuous liquid–liquid extractions for water samples. The general process is the same as for a Soxhlet extractor.

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 is 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 was a gas but has the solvent properties of 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. 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.

24.3.2 Sample Cleanup and Enrichment

A little over 100 years ago, a Russian botanist by the name of M. S. Tswett published a paper describing a new method he had devised for separating out plant pigments by percolating a plant extract through a column of $CaCO_3$ with petroleum ether. He called the process *chromatography*, or "color writing." The fundamental principles of chromatography are the same, regardless of whether it is preparative chromatography or analytical chromatography. There is a stationary phase and a mobile phase, and the separation of sample components is achieved through the differential interaction between the two phases. Compounds that have a higher affinity for the mobile phase will move through the system very quickly, whereas compounds that have a high affinity for the stationary phase will move very slowly through the system (Figure 24.2). This process is the basis for the vast majority of environmental analytical chemistry techniques in use today.

Using one of the extraction techniques described above will remove the analytes of interest from the bulk medium but will also extract other matrix constituents (e.g., wax and lipid inorganic components). These interfering compounds must be removed prior to analysis, and there are various chromatographic methods available to separate the desired components from the matrix interferences.

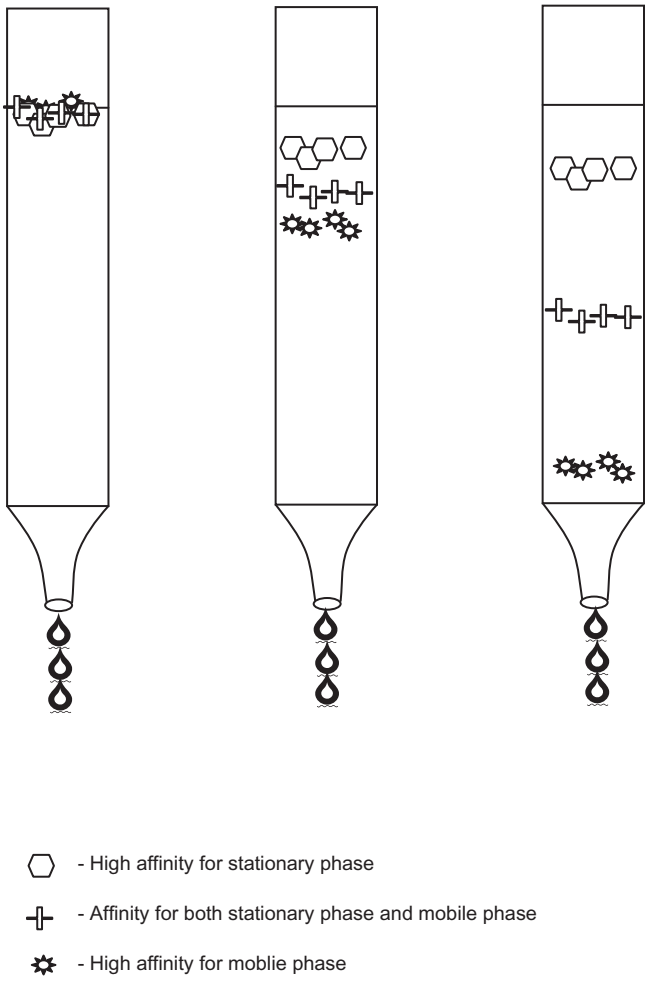

Figure 24.2 Column chromatography. Compounds with high affinity for the mobile phase will be the first to elute from the column.

Thin-Layer Chromatography (TLC) 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 or on resistant plastic 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 constituents 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. This technique is not used much for environmental chemistry applications.

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 quantification (Figure 24.2).

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 0.5–2.0 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 pre-weighed 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 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 the pH of the solution and the isoelectric point of the compounds, compounds of different isoelectric points can be eluted sequentially. Both ionic and anionic exchangers are available. 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.

Size Exclusion Chromatography Also referred to as gel permeation chromatography (GPC), this technique is primarily used during the analysis of biological samples. When tissue samples are extracted with a nonpolar solvent, a good deal of lipids is also extracted. In addition, some configurations of PSDs contain a lipid matrix and would be prepared via GPC as well. GPC columns are packed with a cross-linked polymer material that is very porous. Cross-linked dextrans such as Sephadex or agarose (Sepharose) are commonly used materials. Small molecules can get into the pores and are thus retained for longer periods of time on the column, whereas large molecules (e.g., lipids) cannot and therefore pass through the column very quickly. The GPC material is available in varying pore sizes depending on the application for which it will be used. When using this type of

cleanup, the lipid fraction is retained, dried, and weighed. Thus, analytical results from the sample can be reported on a lipid weight basis where appropriate.

24.3.3 Analysis

Once the samples have been extracted, cleaned up and concentrated, the next step is to analyze the extract using a variety of techniques, depending on the analytes of interest.

Gas–Liquid Chromatography Gas–liquid chromatography, generally referred to as gas chromatography (GC), is used most commonly for the separation and quantification of organic toxicants. This system consists of an injector port, oven, detector, amplifier (electrometer), and supporting electronics (Figure 24.3). Gas chromatographs use a capillary column to effect separation of complex mixtures of organic molecules. The stationary phase is coated onto the inside of the capillary column. The mobile phase in this system is an inert gas (called the carrier gas), usually helium or nitrogen, that passes through the column. The term "gas–liquid chromatography" derives from the fact that the polymer coating that acts as the stationary phase is technically a liquid.

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 column is contained within an oven that can be programmed by the analyst. Similar to the way the solvent systems can be changed in column chromatography, the temperature program can be altered to

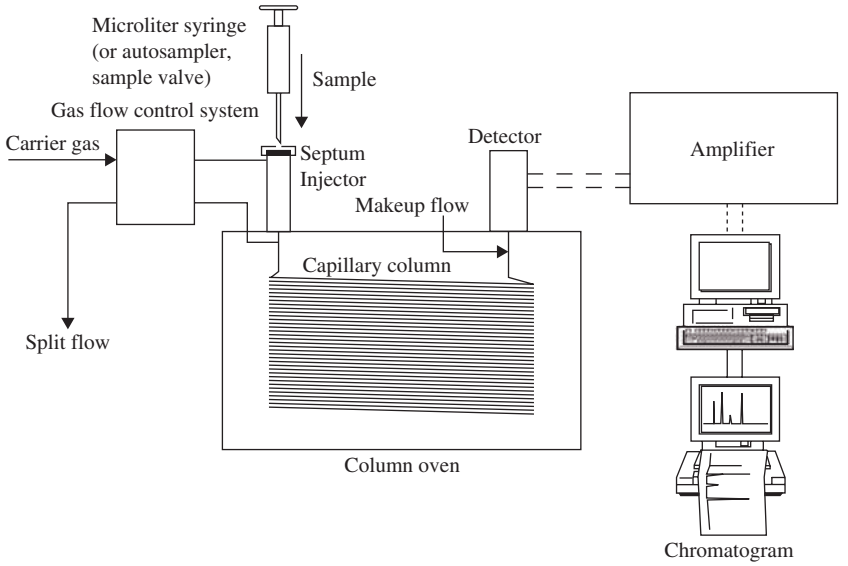

Figure 24.3 Diagram of a typical GC system. Reprinted with permission from *Capillary Gas Chromatography,* D. W. Grant, New York: Wiley, 1996.

maximize the analyte separation while minimizing the run time per sample. The electronic signal produced as the component passes through the detector is amplified by the electrometer, and the resulting signal is sent to a computer or other electronic data-collecting devices for quantification. The time at which a specific compound exits the column for a given set of conditions within the instrument is called the retention time. Standard mixtures are run under the given conditions to determine the retention time for each analyte of interest. This is then used to compare with the retention time of peaks in the unknown samples.

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 is generally made of fused silica 5–60 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 stationary phase. The carrier gas flows through the column at rates of l–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, which 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.

Detectors Five detectors are used widely in toxicant detection: flame ionization detector (FID), nitrogen–phosphorous detector (NPD), flame photometric detector (FPD), electron capture detector (ECD), and mass spectrometer (MS) 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 NPD detects the presence of nitrogen- and phosphorous-containing compounds and functions similarly to an FID. In the NPD, a heated rubidium silicate bead emits ions when nitrogen and phosphorus-containing compounds pass over it, and this signal is passed 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.

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 the 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 and can be used for ultra-trace detection of compounds such as Polychlorinated biphenyls (PCBs) and other chlorinated hydrocarbons.

Although the detectors mentioned above have some degree of specificity (e.g., they detect halogenated compounds or nitrogen-containing compounds), they are nonspecific detectors in that they cannot identify what the compound is, only that it shares a retention time with a specific analyte from the standard. Occasionally, this is remedied through the use of dual-column confirmation methods. In this technique, the same sample is run on two different columns, thus producing two different retention times. In this way, the unknown compound can be confirmed. However, the only truly specific detector is the MS. This detector, discussed in more detail below, bombards the molecule and breaks it apart. The resulting fragments are separated within the detector based on their mass-to-charge ratio (m/z). Thus, regardless of retention time, the mass spectrum of a given compound is unique, much like a fingerprint.

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. However, the most important advance was the development of the MS detector that could be coupled with HPLC.

The instrument consists of a solvent reservoir, a gradient-forming device, a high-pressure pumping device, an injector, a column, and a detector (Figure 24.4). The principle of operation is very similar to that of GC except that the mobile phase is a liquid instead of a gas. The composition of the mobile phase and its flow rate affect separations (recall that in the GC, the oven temperature was controlled to affect separation). 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 15–25 cm in length, with small diameters. (~4.6 mm number diameter). A high-pressure pump is required to force the solvent through this type of column. The major detectors presently used for HPLC are UV or fluorescent spectrophotometers as well as mass spectrometers.

Capillary Electrophoresis (CE) A relatively new analytical technique, CE, is receiving considerable attention in the field of toxicology, 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, a high-voltage power supply, two buffer reservoirs, the capillary (approximately 70 cm × 75 μm in diameter) and a detector (Figure 24.5a). The versatility of the process lies in the ability to separate compounds of interest by a number of modes, including affinity, charge/mass ratios,

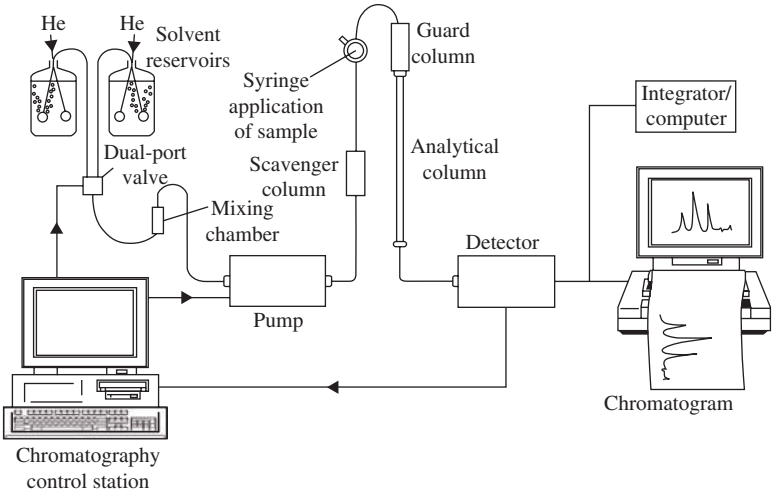

Figure 24.4 High-performance liquid chromatography (HPLC) system. Reprinted with permission from *Chromatographic Methods*, A. Braithwaite and J. F. Smith, New York: Springer, 1996.

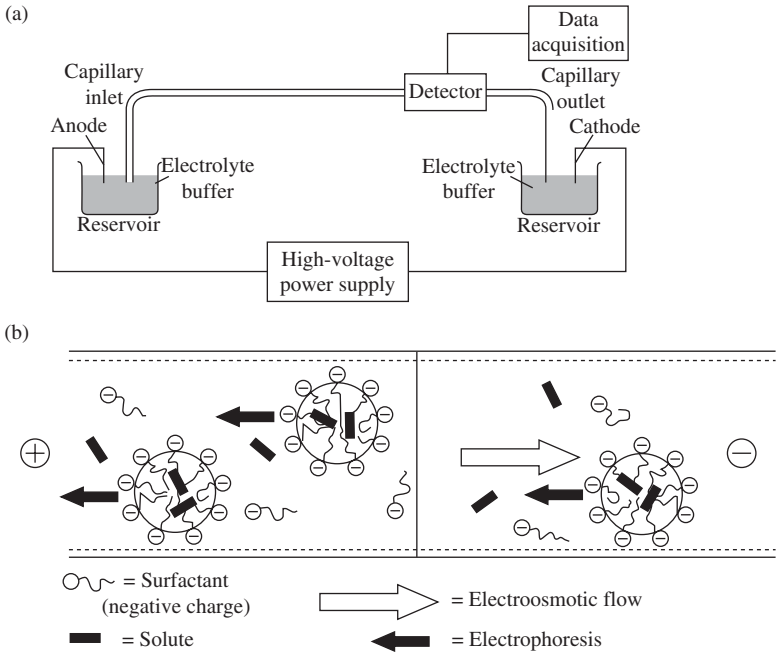

Figure 24.5 (a) CE system and (b) MEKC chromatography. Reprinted with permission from *Instant Notes: Analytical Chemistry*, D. Kealy and P. J. Haines, New York: Garland Science/Taylor & Francis, 2002.

chirality of the 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 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 (MEKC) (Figure 24.5b). Many of these analyses can be carried out in 5–10 min with sensitivities in the low parts-per-billion range. A UV detector is usually used, but greatly sensitivities can be obtained using laser-induced fluorescence detectors.

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, ν, and the quantum energy can be calculated by the equation $E = h\nu$, 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, atomic emission spectroscopy, inductively coupled plasma (ICP), and mass spectroscopy (MS) follow.

AA 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 (flame AA) or by carbon rod atomization in a graphite cup or tube (graphite furnace 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 graphite furnace 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. The disadvantage of AA methods is that only one metal is analyzed at a time.

ICP Spectrometry An even more sensitive instrument has been developed to detect and quantitate, simultaneously, all inorganic species contained with a sample

matrix. With ICP, a stream of argon passed through an induction coil, producing temperatures of up to 10,000 K. When the sample is heated, an emission spectrum is given off and the spectral lines are observed. One such system is the inductively coupled plasma–optical emission spectrometer (ICP-OES). 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 spectra are captured by a charge-coupled device (CCD) that converts the light to measurable electrons at specific wavelengths. Wavelength coverage ranges from 175 to 785 nm. In addition, the ICP can be coupled with 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. In toxicant analysis, MS is widely used as a highly sensitive detection method for GC and is increasingly used with HPLC, CE, and ICP because these instruments can be interfaced to the mass spectrometer. Chromatographic techniques (e.g., GC, 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 quadrapole (Figure 24.6) and the ion trap. Both produce

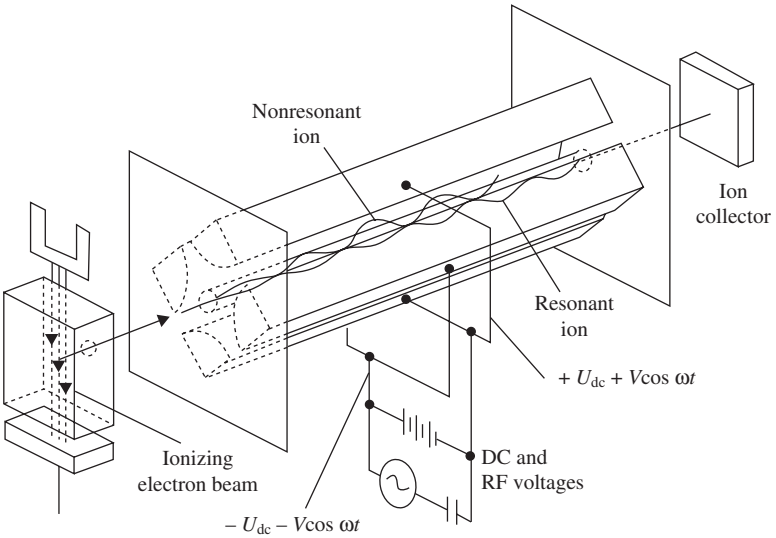

Figure 24.6 Quadrupole mass spectrometer. Reprinted with permission from *Instant Notes: Analytical Chemistry*, D. Kealy and P. J. Haines, New York: Garland Science/Taylor & Francis, 2002.

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.

Bimolecular Interaction Analysis–Mass Spectrometry (BIA-MS) A 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, quantify 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). 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 quantified 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.

24.4 QUANTIFICATION, QA, AND QC

Toxicants are generally found at low concentrations (e.g., parts per million or parts per billion) regardless of the sample matrix being evaluated. These concentrations are based on the measurement of a response from some instrument to the compound(s) of interest from an extract of the sample matrix. Thus, it is necessary to have a system capable of measuring the compound of interest, and in order to ensure the reliability of the data, the analytical process (instrument and analytical method) must be monitored closely.

This measurement process involves much more than injecting some amount of the extracted sample and comparing its response to that of a standard of known concentration. Analytical standards must be prepared, weighed, and diluted carefully to ensure that the concentrations reported reflect those found in the sample analyzed. In addition, the analytical instrument used must be calibrated properly to ensure accuracy. Essentially, this involves two processes: (1) calibration of the detector against the compound of interest in order to eliminate or to minimize any deviation (bias) of response in one direction or another from that expected from previous experience or expected results and (2) calibration of the total analytical system using statistical approaches to minimize bias in determining the presence or absence of the analyte being sought.

24.4.1 Quantification Approaches and Techniques

Analytical Instrument Calibration In setting up instrument parameters, consider what is involved in determining residue levels of an analyte. The data

produced are only as good as the extract derived from the original sample. If the analyte is distributed uniformly over the area sampled, the concentrations found will be equal, regardless of where the sample is taken. Along these same lines, the analytical procedure will result in uniform residue values if all procedures and instrument parameters remain the same. Based on experience, we know that this distribution of residue over an area will vary as will the analytical procedures and instrument parameters. If we increase the number of samples collected and analyzed, the differences observed will tend to get smaller, resulting in a mean or average value that locates the center of the distribution. Ideally, this distribution is called a normal distribution or Gaussian distribution, and looks like a bell (the classic "bell-shaped curve") when the parameters being measured are plotted on a graph (i.e., frequency vs. concentration). Second, the difference in individual measurement, called the standard deviation (σ), defines the variation found in individual measurements. Equations and tables have been developed to determine the significance of suspected deviations and are used to confirm the presence of a suspected problem. If an infinite number of samples from the area are collected and analyzed, the variation in 95% of the samples will cover the true population percentage.

Quantification Quantification of unknown concentrations of COPCs is the ultimate goal of the residue chemist. Residue analysis involves the removal of the compound of interest from some sample matrix. As discussed previously, the sample is collected in the field, and that matrix is then homogenized, subsampled, extracted, cleaned up, concentrated, and finally run on an appropriate instrument. Analytical instruments are calibrated such that they give a consistent reproducible response for a given mass of analyte. The most fundamental decision made is whether the analyte is present or absent, particularly when its concentration is at or close to its detection limit. Because the measurements are derivations of a known relationship between the analyte concentration and the magnitude of the signal made by the instrument, there is an additional signal (noise) generated from the presence of co-extractives, column bleed, and the like. The analyst uses this "contaminated" signal to decide whether the analyte is present or absent and selects one of these choices. The decision process is subject to two types of errors: the analyte is present when actually it is not, and the analyte is absent when actually it is present. The terminology for these decision processes are commonly called "false positives" and "false negatives," respectively. As the mass of analyte increases, the analyst moves beyond simple presence/absence determinations to quantification of concentrations. Using a set of standards to establish a three- or five-point calibration curve, the mass of analyte in the unknown can be extrapolated from the detector response (Figure 24.7).

24.4.2 QA and QC

Over the last 20 years, the reliability of data produced by analytical laboratories has increased dramatically. Strict requirements have ensured that the data were produced under defined standards of quality with a stated level of confidence. The routine day-to-day activities (e.g., matrix fortifications) to control, assess, and ensure the quality of generated data are the QCs associated with analytical processes.

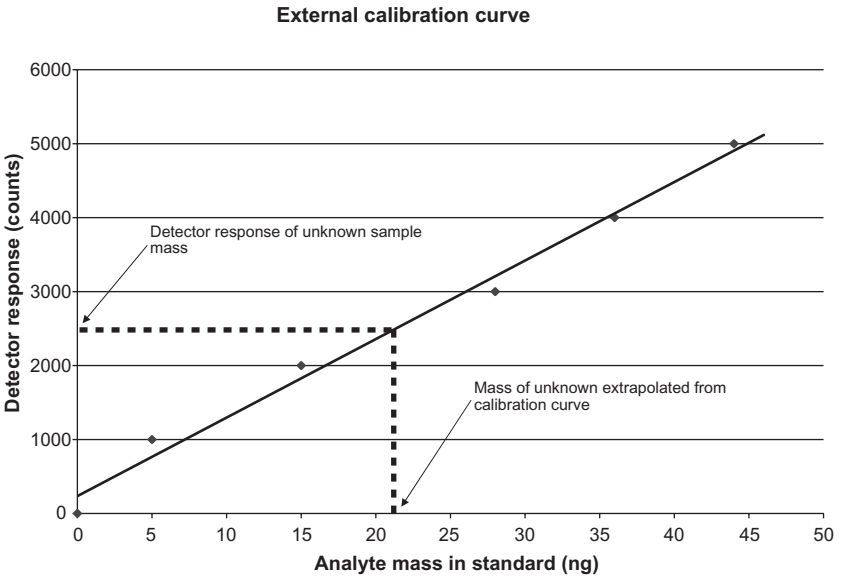

Figure 24.7 A five-point calibration curve. Unknown analyte concentrations are determined by extrapolating from the detector response to the mass.

The management of the system that ensures that these processes are in place and functional is the QA portion of the laboratory program to produce reliable data.

QA is an essential part of analytical protocols. Each laboratory is required to detect and to correct problems in analytical processes and to reduce errors to agreed-upon limits. To produce data that have acceptable quality, all laboratory members must follow established guidelines and protocols. Some of the essential elements that must be included in a QA program are as follows:

1. Laboratory practices (e.g., glass washing protocols) must be developed, reviewed, and updated with the staff's participation on a scheduled basis and followed strictly by all laboratory members.
2. Standard operating procedures (SOPs) (e.g., SOPs monitoring freezer temperatures daily) must be standardized, documented, and supplied to each member of the laboratory staff and updated on a set schedule.
3. Monitoring programs (e.g., surface water monitoring of supplies furnishing public drinking water) must be carefully designed.
4. Maintenance of equipment and instruments must be documented in a laboratory information management system (LIMS) or appropriate maintenance books kept with the equipment.
5. Expiration dates of analytical standards, chemicals, and solvents must be observed and replacements made prior to their expiration date.
6. Good laboratory practices (GLPs) must be implemented as needed.
7. Audits must be performed on a scheduled basis to verify that all aspects of the QA program are operating sufficiently.

QC concerns procedures that maintain a measurement system in a state of statistical control. This does not mean that statistics control the analytical procedures but that statistical evidence is used to ensure that the procedure is working under the conditions set by protocol. The accuracy of an analytical method depends on statistical control being conducted prior to determining any other parameter. How well the basic method will work with the sample matrix being evaluated will depend on the way the QC samples are examined. A comprehensive QC analytical procedure would include the following:

1. Replicated environmental samples to test the precision of the sampling or analytical procedures
2. Replicated analyses conducted on the same sample multiple times in order to determine analytical precision
3. Trip blanks to determine if contaminants are introduced to the processes of collecting, shipping, or storing of samples
4. Matrix-fortified laboratory blanks consisting of solvent and reagent blanks to determine levels of bias due to matrix effects or analytical method problems
5. Sample blanks (a sample matrix that does not contain the toxicant, although this is sometimes difficult to obtain) to ensure no extraneous or interfering peaks; the peaks indicate where a problem might exist in the method used
6. Fortified field blanks to determine the effects that the matrix might have on analyte recovery

Although many of these above procedures may seem mundane, when data are required for decision making, especially in the area of regulatory actions or risk to human health, it is imperative that the data are as reliable and accurate as possible.

24.5 SUMMARY

Whether the exposure analysis is being conducted for a human health or ecological risk assessment, the essential elements are the same: defining the research goal(s), identification of appropriate techniques and methods, development of a sampling scheme to obtain representative samples, isolating the compound(s) of interest, removing potentially interfering components, and quantifying and evaluating the data in relation to the original research goals. These essential elements are critical to ensuring that the data generated answer what is requested and enable some decision affecting environmental or human welfare. Essential decision criteria must be included in the protocol that describes the analytical process in detail, including the objective(s) of the study, the QA/QC requirements, the sample plan, methods of analysis, calculations, documentation, and data reporting. Meaningful data can only be generated with the proper method of analysis.

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SAMPLE QUESTIONS

1. Describe the basic principles behind chromatographic separation.

2. Name a sampling scheme and describe when one might use it.

3. What are the steps involved in the process of evaluating contamination in the environment?

4. What are three of the essential elements that should be included in a laboratory QA program?

CHAPTER 25

Basics of Environmental Toxicology

GERALD A. LEBLANC and DAVID B. BUCHWALTER

25.1 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. Gases quickly dispersed into the atmosphere; liquids were diluted into receiving waters and were 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 U.S. Fish & Wildlife Service, Rachel Carson, published a book that began by describing a world devoid of birds and from which the title *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 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 United States 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. Though this definition would encompass toxic chemicals naturally found in the environment (i.e., animal venom and microbial and plant toxins), environmental toxicology is typically associated with the study of environmental chemicals of anthropogenic origin. Environmental toxicology can be divided

A Textbook of Modern Toxicology, *Fourth Edition*. Edited by Ernest Hodgson

into two subcategories: 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 (i.e., fish and wildlife). 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 a myriad of organisms that makes up 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 26) 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 27.

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.

25.2 ENVIRONMENTAL PERSISTENCE

Many abiotic and biotic processes that function in concert to eliminate (i.e., degrade) toxic chemicals exist in nature. 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 hazards (e.g., dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls [PCBs], 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)) resist degradative processes and accordingly persist in the environment for extremely long periods of time (Table 25.1). Trace metals represent an extreme case of persistence because as elements, metals cannot be broken down in the environment. 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

TABLE 25.1 Environmental Half-Life of Some Chemical Contaminants

Contaminant	Half-Life	Media
DDT	10 years	Soil
TCDD	9 years	Soil
Atrazine	25 months	Water
Benzoperylene (polycyclic aromatic hydrocarbon (PAH))	14 months	Soil
Phenanthrene (PAH)	138 days	Soil
Carbofuran	45 days	Water

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 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. Estuarine sediments in the United Kingdom are contaminated with metals that date back to tin mining by the Romans. Both biotic and abiotic processes contribute to the degradation of chemicals.

25.2.1 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, on foliar surfaces, or in shallow 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 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 25.1.

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 (e.g., parathion, Figure 25.1), 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.

25.2.2 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 (bacteria, archaea, and fungi) most frequently degrade organic and inorganic compounds by using them as electron donors, electron acceptors, or as sources of nutrients such as nitrogen or sulfur. 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

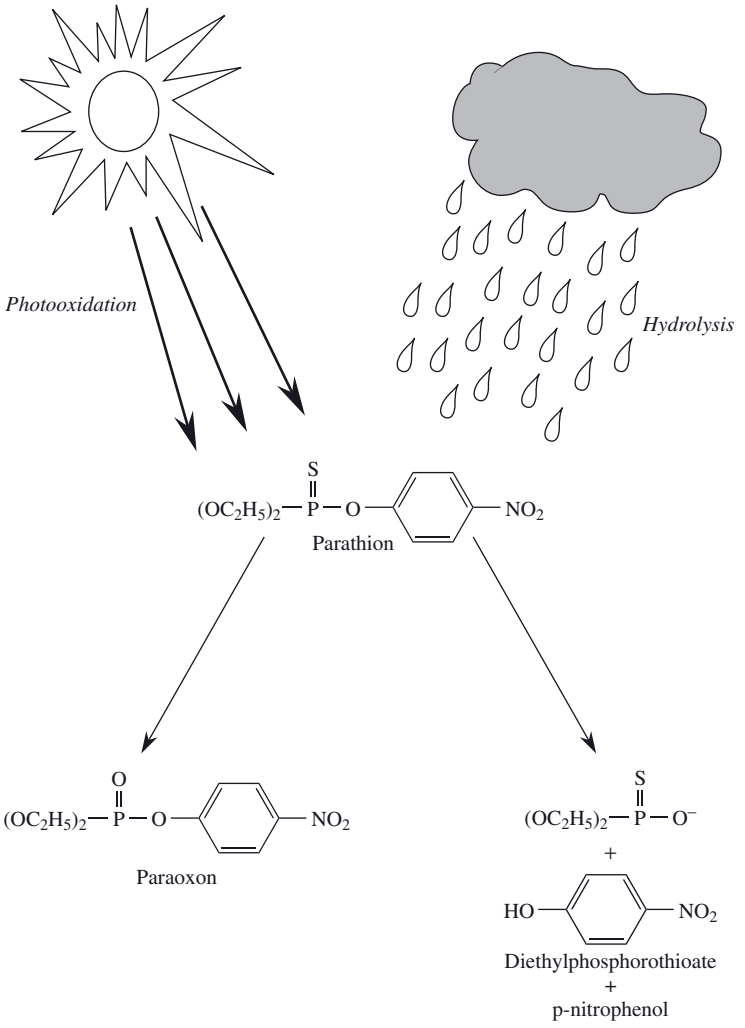

Figure 25.1 The effect of sunlight (photooxidation) and precipitation (hydrolysis) on the degradation of parathion.

degradation (e.g., hydrolysis and 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.

25.2.3 Nondegradative Elimination Processes

Many processes that contribute to the regional elimination of a contaminant by altering its distribution are operative in the environment. Contaminants with sufficiently high vapor pressure can evaporate from contaminated terrestrial or

aquatic compartments and can 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 contributes to contaminant redistribution. Sorption of contaminants to suspended solids in an aquatic environment with commensurate sedimentation can result with the removal of the contaminants from the water column and their 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 organisms. Often the amount of organic carbon in sediments is tied to the bioavailability of contaminants. Due to its high affinity to many metals, sulfur can affect metal bioavailability in sediments as well. 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 26.

25.3 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 5). 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). Many organic environmental chemicals are largely taken up by organisms by passive diffusion. Metals, on the other hand, cannot simply diffuse across plasma membranes. Instead, metals are usually transported by ion pumps or channels that otherwise would transport essential ions such as calcium or sodium. 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 organic chemicals must traverse the lipid bilayer of membranes to enter the body, the 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 (a) lakes, rivers, and oceans serve as sinks for these chemicals; and (b) 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 than the concentration of the chemical found in the environment (Table 25.2). The degree to which aquatic organisms accumulate xenobiotics from the environment is largely dependent upon the lipid content of the organism, since body lipids serve as the primary site of retention of the chemicals (Figure 25.2).

TABLE 25.2 Bioaccumulation of Some Environmental Contaminants by Fish

Chemical	Bioaccumulation Factor[a]
DDT	127,000
TCDD	39,000
Endrin	6800
Pentachlorobenzene	5000
Lepthophos	750
Trichlorobenzene	183

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

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

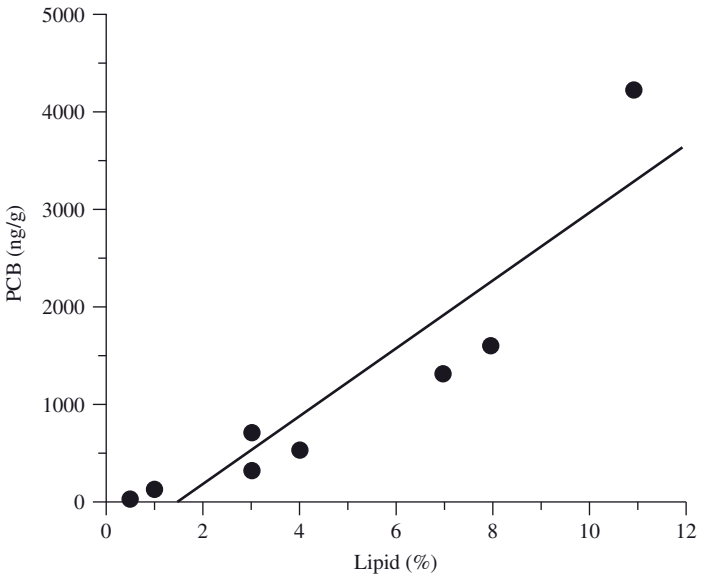

Figure 25.2 Relationship between lipid content of various organisms sampled from Lake Ontario and whole-body PCB concentration. Data derived from Oliver, B. G. and A. J. Niimi. *Environ. Sci. Technol.* **22**:388–397, 1988.

Most aquatic animals directly exchange ions with the surrounding water to maintain salt–water balance. Since many metals have affinities for ion transport systems (usually located on gills or other body surfaces), direct uptake of metals from solution can be an important route of exposure. Freshwater animals are considerably "saltier" than surrounding water, creating a situation where the tendency is to accumulate body water and to lose salts diffusively. (Diffusive salt loss occurs through paracellular channels, not across plasma membranes.) Therefore, freshwater animals are constantly faced with the task of excreting excess body water and of sequestering salts from the surrounding water. Very few freshwater animals drink the surrounding water. Saltwater animals face the opposite challenge. They must conserve their body water and excrete excess salts. To accomplish this, many saltwater organisms drink the surrounding water and produce a small volume of concentrated salty urine. Still, there is direct uptake of ions from the surrounding water to help compensate for salts lost in urine production.

Both organic chemicals and metals 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 25.3, 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 prey relative to that found in the abiotic environment. For many compounds, 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. For some elements such as selenium, bioaccumulation is thought to be primarily derived from the diet. 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. Methyl mercury and organic selenium species are examples of organometallic compounds that tend to biomagnify in food webs.

For many trace metals, there appears to be a major difference between fish and invertebrates in bioaccumulation patterns. Invertebrates tend to have very high assimilation efficiencies for metals from their diets, making the dietary route of exposure very important to understand in this group. Fish, on the other hand, tend to have poor assimilation efficiencies for many metals. This difference has practical implications in the setting of water quality criteria, because the susceptibility of invertebrates to metals is likely underestimated from toxicity tests that use only dissolved exposures.

Bioaccumulation of lipohilic compounds 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 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 the mortality of adult organisms as they approach reproductive maturity. Lipophilic chemicals also can be transferred to the offspring in lipids associated with the yolk of oviparous organisms or with the milk of mammals, resulting in toxicity to the offspring that was not evident in the parental organisms.

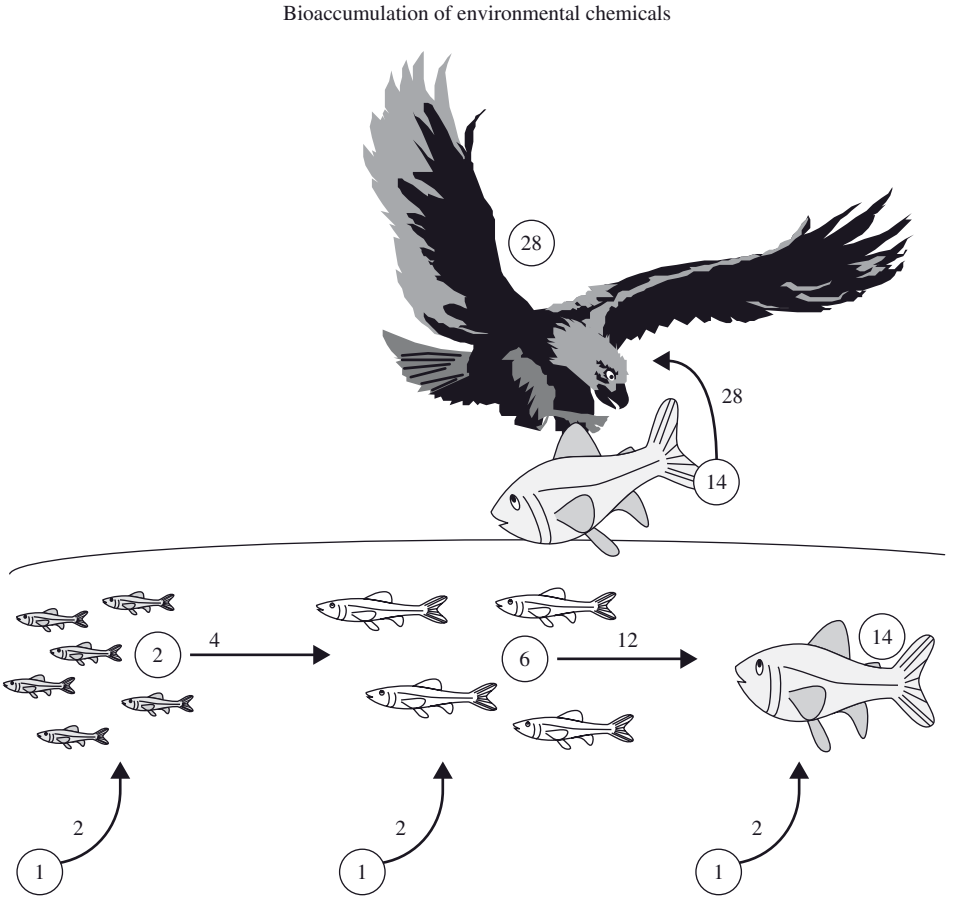

Figure 25.3 Bioaccumulation of a chemical along a generic food chain. In this simplistic paradigm, the amount of the chemical in the water is assigned an arbitrary concentration of 1, and it is assumed that the chemical will bioaccumulate either from the water to the fish or from one trophic level to 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.

25.3.1 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 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 (e.g., 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

TABLE 25.3 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	4900	780
Tris(2,3-dibromo-propyl)phosphate	High	4570	3

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

example, sorption of benzo[a]pyrene to humic acids reduced its propensity to bioaccumulate in sunfish by a factor of 3. 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 6) are rendered more water soluble and less lipid soluble. The biotransformed chemical is thus less likely to be sequestered in lipid compartments and is more likely to be eliminated from the body. As depicted in Table 25.3, chemicals that are susceptible to biotransformation bioaccumulate much less than would be predicted based upon lipophilicity. Conjugation of xenobiotics to glutathione and glucuronic acid (Chapter 6) can target the xenobiotic for biliary elimination through active transport processes, thus greatly increasing the rate of elimination (Chapter 9). Differences in chemical elimination rates contribute to species differences in bioaccumulation.

25.4 TOXICITY

25.4.1 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 (e.g., derailment of a train resulting in leakage of a chemical into a river) or with imprudent use of the chemical (e.g., 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 10, the acute toxicity of a chemical is commonly quantified as the median lethal concentration (LC_{50}) or median lethal dose (LD_{50}). These measures do not provide any insight into the environmentally acceptable levels of contaminants (a concentration that kills 50% of the exposed organisms is hardly acceptable). However, LC_{50} and LD_{50} values do provide statistically sound, reproducible measures of the relative acute toxicity of chemicals. LC_{50} and LD_{50} ranges for aquatic and terrestrial wildlife, respectively, and their interpretation are presented in Table 25.4.

TABLE 25.4 Ranking Scheme for Assessing the Acute Toxicity of Chemicals to Fish and Wildlife

Fish LC_{50} (mg/L)	Avian/Mammalian (LD_{50}, 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

Acute toxicity of environmental chemicals is determined experimentally with select species that serve as representatives of particular levels of trophic organization within an ecosystem (e.g., mammal, bird, fish, invertebrate, vascular plant, algae). For example, the United States 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 with respect to 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.

25.4.2 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 10 for more detail on cholinesterase inhibition). An inhibition of 40–80% 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 non-moribund 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 but rather accumulate in the lipid phase or the lipid–aqueous interface of membranes at sufficient levels to disrupt membrane function. Chemicals that induce narcosis include alcohols, ketones, benzenes, ethers, and aldehydes.

Osmoregulatory Disturbance Some metals have high affinity for ion transport systems. For example, cadmium and zinc have high affinity for calcium transport systems, whereas silver has a high affinity for sodium transporters. If the dissolved concentrations of these metals are high enough, it can lead to ionoregulatory disruption and acute toxicity.

Physical Effects Perhaps the 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 result in the coating of animals, such as birds and marine mammals, which 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 seabirds 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.

25.4.3 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 sufficiently

TABLE 25.5 Acute and Chronic Toxicity of Pesticides Measured from Laboratory Exposures of Fish Species

Pesticide	LC_{50} (μg/L)	Acute Toxicity	Chronic Value (μg/L)	ACR	Chronic Toxicity
Endosulfan	166	Extremely toxic	4.3	39.0	Yes
Chlordecone	10	Extremely toxic	0.3	33.0	Yes
Malathion	3000	Very toxic	340.0	8.8	No
Carbaryl	15,000	Moderately toxic	378.0	40.0	Yes

high doses, 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 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 LC_{50} value by the CV. Chemicals that have an ACR of less than 10 typically have low-to-no chronic toxicity associated with them (Table 25.5).

The mechanisms underlying the chronic toxicity of metals are poorly understood, but three major possibilities exist. First, many metals directly generate reactive oxygen species or scavenge thiols that help mediate oxidative damage in the cell. Second, because of the affinity of some metals to sulfur, and the importance of sulfur in many proteins, exposure to some metals may affect protein tertiary structure and function. Third, the displacement of essential metals in metalloenzymes with exogenous toxic metals can render them ineffective and can cause toxicity. This third mechanism is ripe for further study, as metals play a role in approximately one-third of all known enzymes.

The following must always be considered when assessing the chronic toxicity of a chemical:

A. Simple numerical interpretations of chronic toxicity based upon ACRs serve only as gross indicators of the potential chronic toxicity of the chemical. Laboratory exposures designed to establish CVs most often focus upon a few general end points such as survival, growth, and reproductive capacity. Examination of more subtle end points of chronic toxicity may reveal significantly different CVs.
B. 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.
C. 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.

TABLE 25.6 Toxicity of Tributyltin to Aquatic Organisms

Species	Acute Toxicity (LC_{50}, 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

25.4.4 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 190 species of neogastropods. Imposex has been implicated in the 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 laboratory at low parts-per-billion concentrations (Table 25.6). However, exposure of neogastropods to low parts-per-trillion concentrations can cause imposex (Table 25.6). Thus, neogastropods are uniquely sensitive to the toxicity of tributyltin with effects produced that were not evident in standard laboratory toxicity characterizations.

25.4.5 Abiotic and Biotic Interactions

Chlorofluorocarbons-Ozone-ultraviolet radiation-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, some 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 the 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 or

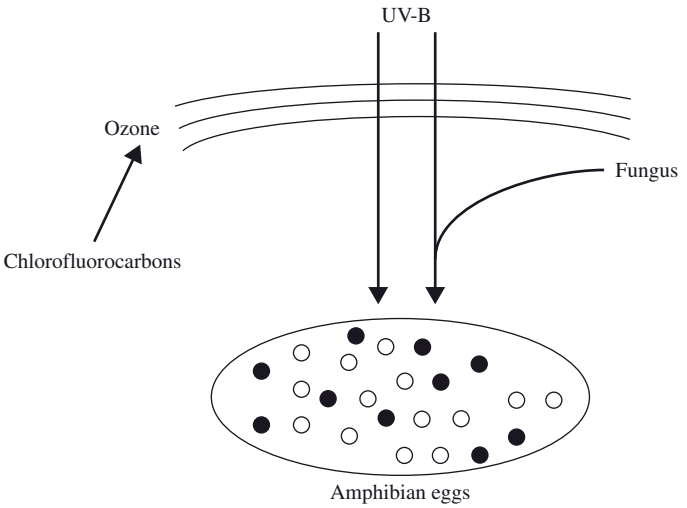

Figure 25.4 Abiotic and biotic interactions leading to the indirect toxicity of chlorofluorocarbons to amphibians. The atmospheric release of chlorofluorocarbons causes the depletion of the stratospheric ozone layer (abiotic–abiotic interaction). Depleted ozone allows for increased penetration of UV-B radiation (abiotic–abiotic interaction). UV-B radiation alone and in combination with fungus (abiotic–biotic interaction) cause increased mortality of amphibian embryos.

disease organisms 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 >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 25.4). 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 Pulp Mill 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

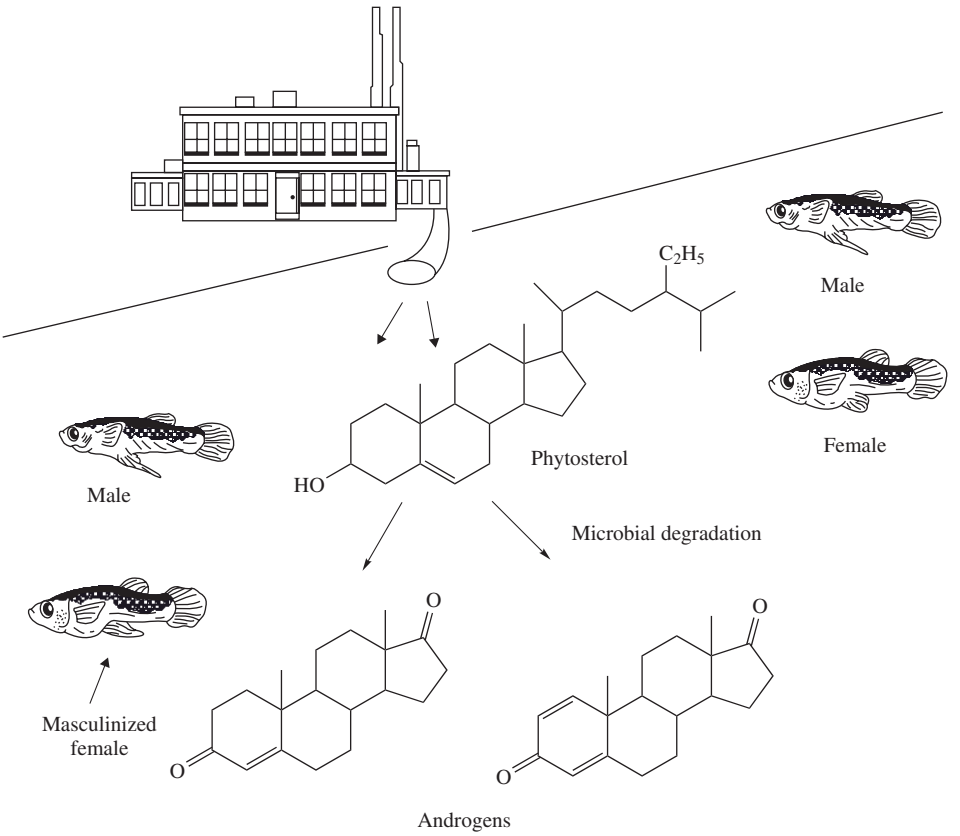

Figure 25.5 Indirect toxicity of kraft pulpmill effluent to mosquito fish. Phytosterols in the mill effluent are converted to C19 steroidal androgens through the action of microorganisms in the environment. These androgens masculinize both anatomy and behavior of female mosquito fish. An arrow identifies the modified anal fin on the masculinized female.

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 pulp mill 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 pulp mill effluent can be converted to androgenic C19 steroids by microorganisms, and these steroids are capable of masculinizing female fish (Figure 25.5). Thus, abiotic (phytosterols)–biotic (microorganisms) interactions in the environment must occur before this occult toxicity associated with the kraft pulp mill effluent is unveiled.

Environmental Contaminants and Disease among Marine Mammals Worldwide, massive mortality has occurred over the past 20 years among populations of harbor seals, bottlenose dolphins, and other marine mammals. 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 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 by assessing the integrity of the immune system in the seals. Seals fed with 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.

25.5 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 analyses of the environmental consequences of environmental contamination. Such analyses have resulted in curtailing the release of demonstrated hazardous chemicals into the environment and have provided 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 the 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 was 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 environment do not imply that the role of environmental toxicologists in the twenty-first century will diminish. A dearth of information persists in areas vital to the 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 the 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.

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SAMPLE QUESTIONS

1. What is the major chemical characteristic that is responsible for the bioaccumulation of organic chemicals?

2. What impact does biotransformation have on bioaccumulation? Why?

3. What three characteristics tend to be shared by chemicals that historically have posed toxicological problems in the environment?

4. Is tributyltin a problematic chemical due to its acute toxicity or chronic toxicity? What adverse effect of tributyltin (TBT) occurs at nanogram per liter exposure concentrations?

CHAPTER 26

Transport and Fate of Toxicants in the Environment

DAMIAN SHEA

26.1 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 26.1 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 26.1). 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

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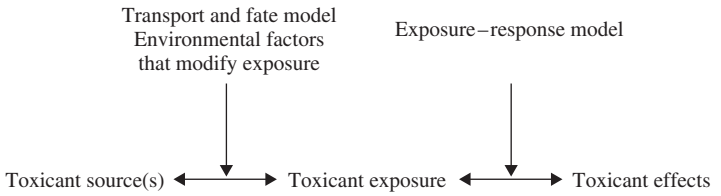


Figure 26.1 Environmental fate models are used to help determine how the environment modifies exposure resulting from various sources of toxicants.

physical–chemical properties, combined with the characteristics of the environment to which it is released.

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 vs. 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.

26.2 SOURCES OF TOXICANTS TO THE ENVIRONMENT

Environmental sources of toxicants can be categorized as either *point sources* or *nonpoint sources* (Figure 26.2). 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 source 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, which 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 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 26.2).

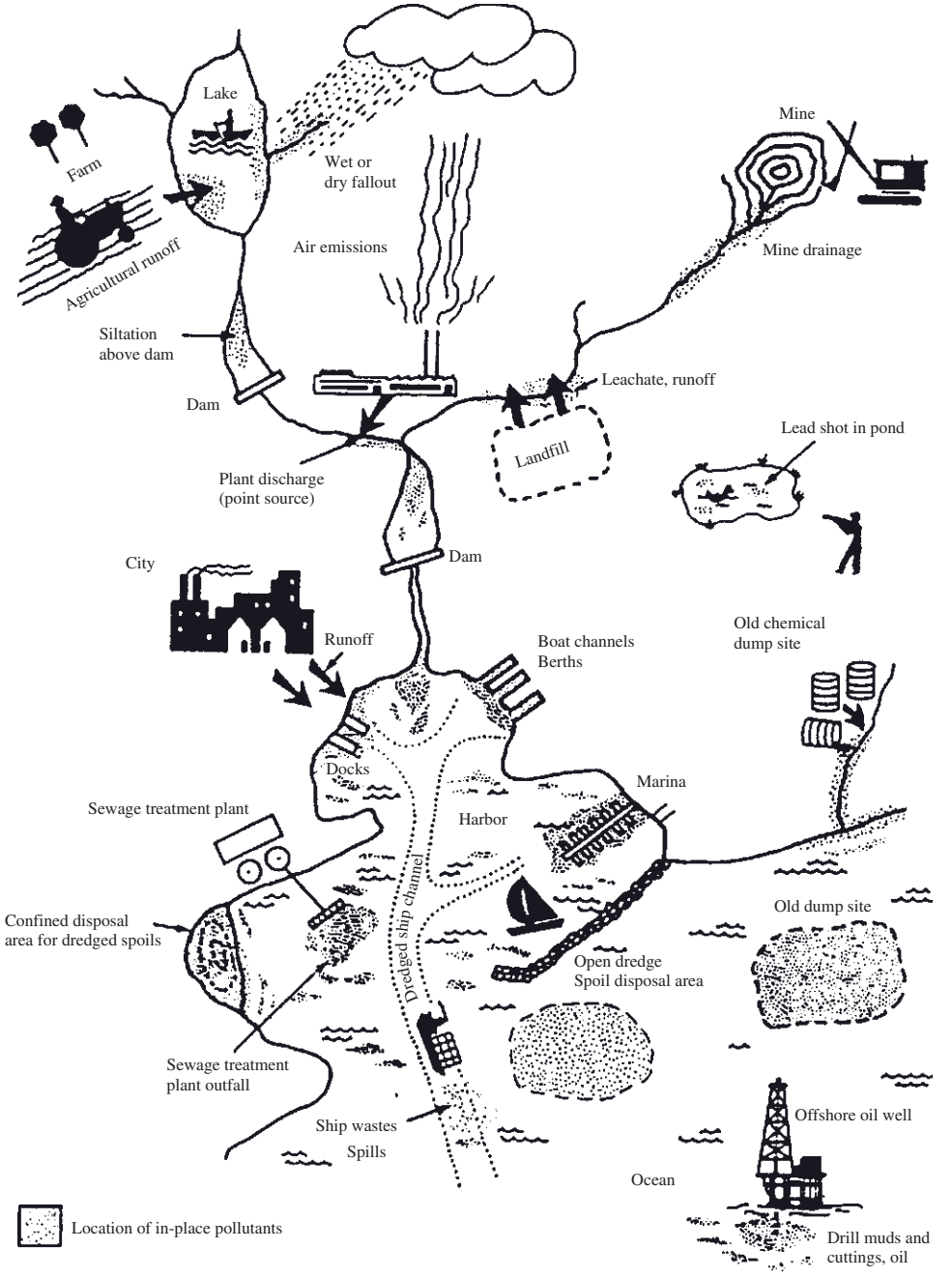


Figure 26.2 Toxicants enter the environment through many point and nonpoint sources.

The rate (units of gram per hour) 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 (gram per cubic meter) and the flow rate of the medium (cubic meter per hour). 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 24). 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 26.3 for the herbicide atrazine. Rainfall following the application of atrazine results in drainage ditch loadings more than 100-fold higher than just 2 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

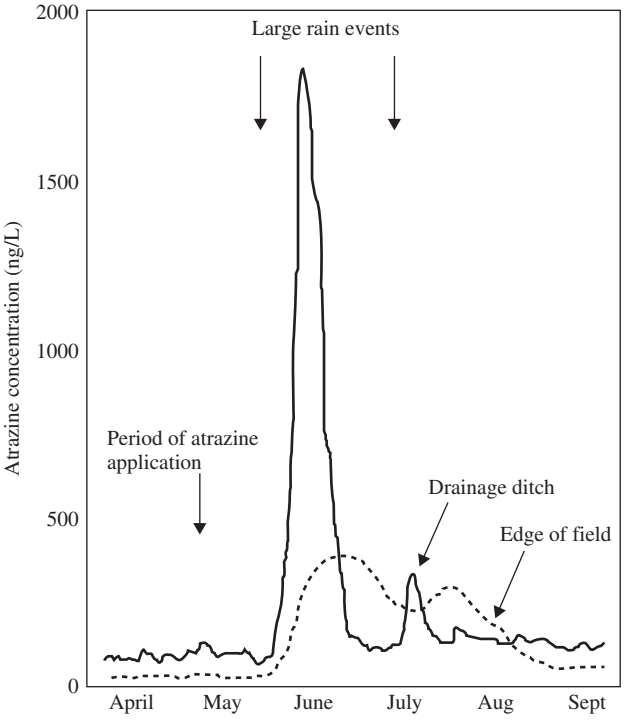


Figure 26.3 The loading of atrazine from an agricultural field to an adjacent body of water is highly dependent on rainfall and on the presence of drainage ditches that collect the chemical and focus its movement in the environment.

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 to model the transport of atrazine from the field.

26.3 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*.

26.3.1 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 with 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 26.3). 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 of time by assuming conservation of mass and by 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. Using 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 would 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 dichlorodiphenyltrichloroethane (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 can 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 also 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 a 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 the earth's surface (dry deposition). Under certain conditions, both processes can be treated as a 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, which is proportional to rainfall intensity.

26.3.2 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):

$$N = -DA(dC/dx),$$

where D is the diffusivity or the 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 gram per square meter hour (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 (gram per hour). In either case, the diffusion equation can be integrated numerically and can even be 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 (meter per hour). The chemical flux is then the product of this velocity, area, and concentration:

$$N = -k_M AC.$$

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 pore water, 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 in 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 the 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, so the term describing the departure from equilibrium (C_1–C_2K_{12}) becomes zero, and thus the net rate of transfer is also 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.

26.4 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:

$$C_1/C_2 = K_{12}.$$

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.

26.4.1 Air–Water Partitioning

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

$$K_{\text{air–water}} = H = C_{\text{air}}/C_{\text{water}},$$

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

$$H' = P_{\text{air}}/C_{\text{water}},$$

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 (such as 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 (such as 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.

26.4.2 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 10), but their affinity for water varies by many orders of magnitude. Thus, it is largely aqueous solubility that 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).

26.4.3 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 that 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. Phytoplanktons 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.

26.4.4 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

$$K_P = C_S / C_W,$$

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). Using this form, K_P has units of liter per kilogram 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

$$K_P = f_{OC} K_{OC},$$

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 1.0, 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 by rearranging to solve for C_W:

$$K_P = C_S / C_W = f_{OC} 0.41 K_{OW}$$
$$C_W = C_S / f_{OC} 0.41 K_{OW}.$$

This last equation forms the basis for the Environmental Protection Agency's sediment quality criteria that are to be 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 both scientists and policymakers.

26.5 TRANSFORMATION PROCESSES

The potential environmental hazard associated with the use of a chemical is directly related to its persistence in the environment (see Chapter 25), 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 can be mediated directly by biota, particularly bacteria.

26.5.1 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

$$K_a = [H_3O^+][A^-]/[HA].$$

For convenience, we often express equilibrium constants as the negative logarithm, or the pK value. Thus, the relative proportion of the neutral and charged species will be a function of the pK_a and solution pH. When the pH is equal to pK_a, equal concentrations of the neutral and ionized forms will be present. When pH is less than the pK_a, the neutral species will be predominant; when pH is greater than pK_a, 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 pK_a 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 pK_a 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

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

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

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

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 to reduce effective exposure. The formation of metal sulfides is important in anaerobic soil and sediment, in stagnant ponds and basins, and in 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 incorporates 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 timescales.

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, using copper as an example, 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 ethylenediaminetetraacetic acid (EDTA) or glutathione (GSH). 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 Le Chatelier's principle as shown here for CuS and OH^-, Cl^-, and GSH:

$$\mathrm{CuS(s) + H^+ = Cu^{2+} + HS^-} \quad K_{sp}$$

$$\mathrm{Cu^{2+} + OH^- = CuOH^+} \quad K_{CuOH}$$

$$\mathrm{Cu^{2+} + Cl^- = CuCl^+} \quad K_{CuCl}$$

$$\mathrm{Cu^{2+} + GSH = CuGS^+ + H^+} \quad K_{CuGS}.$$

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:

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

and the overall equilibrium constant derived as shown:

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

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, which 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 methylmercury by anaerobic bacteria) or less toxic species (oxidation of tributyl tin to elemental tin), or can be temporarily immobilized (e.g., via microbial reduction of sulfate to sulfide, which then precipitates as an insoluble metal sulfide mineral).

26.5.2 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 form a complex with numerous ligands, and later reprecipitate 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), in 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

$$d[\mathrm{RX}]/\mathrm{dt} = k_{\mathrm{obs}}[\mathrm{RX}] = k_{\mathrm{a}}[\mathrm{H}^+][\mathrm{RX}] + k_{\mathrm{n}}[\mathrm{RX}] + k_{\mathrm{b}}[\mathrm{OH}^-][\mathrm{RX}],$$

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

$$k_{\mathrm{obs}} = k_{\mathrm{a}}[\mathrm{H}^+] + k_{\mathrm{n}} + k_{\mathrm{b}}[\mathrm{OH}^-].$$

Neutral hydrolysis is dependent only on water that 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 depends 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

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

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 follow 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 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. Using halogenated hydrocarbons as an example, 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 (5–8), the hydrolysis rate of methoxychlor is invariant, while the hydrolysis of DDT is about 15-fold faster at pH 8 compared with 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 1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE), while the methoxychlor hydrolysis product shifts from the alcohol at pH 5–8 (nucleophilic substitution) to the dehydrochlorinated 1,1-bis(p-methoxyphenyl)-2,2-dichloroethylene (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 auto-oxidation or weathering is commonly used to describe the general oxidative degradation of a chemical (or chemical mixture such as 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 the 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 to 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 may even decrease. This complicates the modeling and prediction of biotransformation rates in nature. When the toxicant concentration (and potential energy) is 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 co-metabolism.

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).

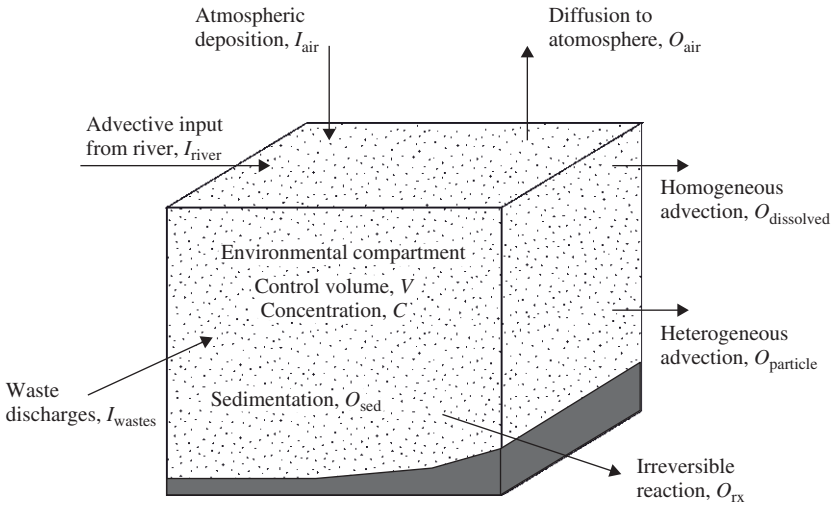
Long-term exposure of microbial populations to certain toxicants often is necessary for adaptation of enzymatic systems capable of degrading those toxicants. This was the case with the *Exxon Valdez* oil spill in Alaska in 1989. Natural microbial populations in Prince William Sound, Alaska, had developed 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, the 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.

26.6 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 26.1). 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 becomes 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



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

$V\mathrm{d}c/\mathrm{d}t = (I_{air} + I_{river} + I_{wastes}) - (O_{air} + O_{sed} + O_{dissolved} + O_{particle} + O_{rx})$

at steady state, $\mathrm{d}c/\mathrm{d}t = 0$ and $(I_{air} + I_{river} + I_{wastes}) = (O_{air} + O_{sed} + O_{dissolved} + O_{particle} + O_{rx})$

Figure 26.4 An illustration of constructing a simple chemical mass balance model.

for advective and diffusive transport processes are written. And lastly, chemical transformation processes are quantified. This model-building process is illustrated in Figure 26.4. 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 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 timescale 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 to 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 a more detailed rate expression is necessary. An illustration of this mass balance approach is given in Figure 26.5 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.

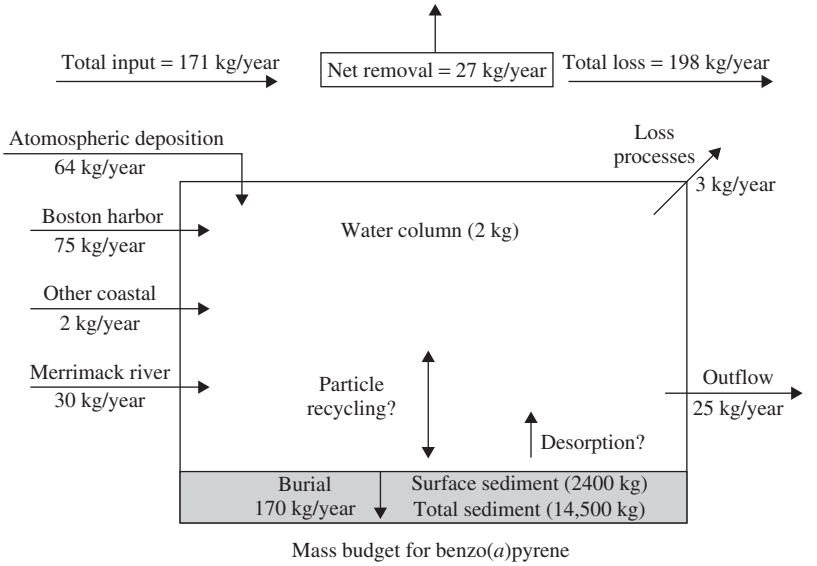


Figure 26.5 An illustration of the information provided by a chemical mass balance model. The annual mass budget of benzo[*a*]pyrene in Massachusetts Bay is shown.

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SAMPLE QUESTIONS

1. Describe how the transport and fate of a chemical fits within the risk assessment paradigm.

2. Describe the difference between point source and nonpoint source pollution and give examples of each.

3. Describe how our knowledge of the behavior of nonpolar chemicals can be used to assess the toxicity of contaminated sediments.

4. List two (or three) types of reversible transformation reactions and give an example of each.
5. List two (or three) types of irreversible transformation reactions and give an example of each.
6. Describe how the knowledge of the source and fate of a chemical can be combined to develop a model to predict exposure in an environmental compartment.

CHAPTER 27

Environmental Risk Assessment

DAMIAN SHEA

27.1 INTRODUCTION

Risk assessment is the process of assigning magnitudes and probabilities to adverse effects associated with an event. The development of risk assessment methodology has focused on accidental events (e.g., an airplane crash) and specific environmental stresses to humans (exposure of humans to chemicals), and thus most risk assessment is characterized by discrete events or stresses affecting well-defined end points (e.g., incidence of human death or cancer). This *single stress–single end-point* relationship allows the use of relatively simple statistical and mechanistic models to estimate risk and is widely used in human health risk assessment. However, this simple paradigm has only partial applicability to ecological risk assessment because of the inherent complexity of ecological systems and the exposure to numerous physical, chemical, and biological stresses that have both direct and indirect effects on a diversity of ecological components, processes, and end points. Thus, although the roots of ecological risk assessment can be found in human health risk assessment, the methodology for ecological risk assessment is not well developed and the estimated risks are highly uncertain. Despite these limitations, resource managers and regulators are looking to ecological risk assessment to provide a scientific basis for prioritizing problems that pose the greatest ecological risk and to focus research efforts in areas that will yield the greatest reduction in uncertainty.

To this end, the United States Environmental Protection Agency (EPA) has issued guidelines for planning and conducting ecological risk assessments. Because of the complexity and uncertainty associated with ecological risk assessment, the EPA guidelines provide only a loose framework for organizing and analyzing data, information, assumptions, and uncertainties to evaluate the likelihood of adverse ecological effects. However, the guidelines represent a broad consensus of the present scientific knowledge and experience on ecological risk assessment. This chapter presents a brief overview of the ecological risk assessment process as presently described by the EPA.

Ecological risk assessment can be defined as

> The process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors.

A Textbook of Modern Toxicology, *Fourth Edition*. Edited by Ernest Hodgson

Estimating the *likelihood* can range from qualitative judgments to quantitative probabilities, though quantitative risk estimates still are rare in ecological risk assessment. The *adverse ecological effects* are changes that are considered undesirable because they alter valued structural or functional characteristics of ecological systems and usually include the type, intensity, and scale of the effect as well as the potential for recovery. The statement that effects *may occur or are occurring* refers to the dual *prospective* and *retrospective* nature of ecological risk assessment. The inclusion of *one or more stressors* is a recognition that ecological risk assessments may address single or multiple chemical, physical, and/or biological stressors. Because risk assessments are conducted to provide input to management decisions, most risk assessments focus on stressors generated or influenced by anthropogenic activity. However, natural phenomena also will induce stress that results in adverse ecological effects and cannot be ignored.

The overall ecological risk assessment process is shown in Figure 27.1 and includes three primary phases: (1) problem formulation, (2) analysis, and (3) risk

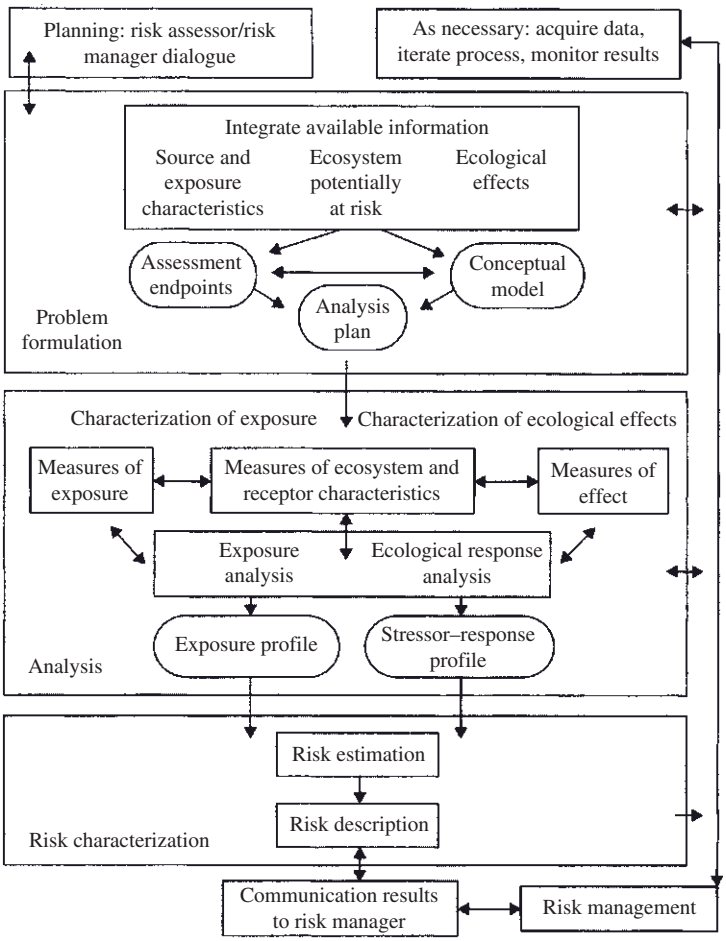

Figure 27.1 The ecological risk assessment framework as set forth by the U.S. Environmental Protection Agency.

characterization. Problem formulation includes the development of a conceptual model of stressor–ecosystem interactions and the identification of risk assessment end points. The analysis phase involves evaluating exposure to stressors and the relationship between stressor characteristics and ecological effects. Risk characterization includes estimating risk through the integration of exposure and stressor–response profiles, describing risk by establishing lines of evidence and by determining ecological effects, and communicating this description to risk managers. While discussions between risk assessors and risk managers are emphasized both at risk assessment initiation (planning) and completion (communicating results), usually, a clear distinction is drawn between risk assessment and risk management. Risk assessment focuses on scientifically evaluating the likelihood of adverse effects, and risk management involves the selection of a course of action in response to an identified risk that is based on many factors (e.g., social, legal, or economic) in addition to the risk assessment results. Monitoring and other data acquisition is often necessary during any phase of the risk assessment process, and the entire process is typically iterative rather than linear. The evaluation of new data or information may require revisiting a part of the process or conducting a new assessment.

27.2 FORMULATING THE PROBLEM

Problem formulation is a process for generating and evaluating preliminary hypotheses about why ecological effects have occurred, or may occur, because of human activities. During problem formulation, management goals are evaluated to help establish objectives for the risk assessment; the ecological problem is defined; and the plan for analyzing data and characterizing risk is developed. The objective of this process is to develop (1) assessment end points that adequately reflect management goals and the ecosystem they represent and (2) conceptual models that describe critical relationships between a stressor and assessment end point or among several stressors and assessment end points. The assessment end points and the conceptual models are then integrated to develop a plan or proposal for risk analysis.

27.2.1 Selecting Assessment End Points

Assessment end points are *explicit expressions of the actual environmental value that is to be protected* and they link the risk assessment to management concerns. Assessment end points include both a valued or key ecological entity and an attribute of that entity that is important to protect and that is potentially at risk. The scientific basis for a risk assessment is enhanced when assessment end points are both ecologically relevant and susceptible to the stressors of concern. Assessment end points that also logically represent societal values and management goals will increase the likelihood that the risk assessment will be understood and used in management decisions.

Ecological Relevance Ecologically relevant end points reflect important attributes of the ecosystem and can be functionally related to other components of the ecosystem; they help sustain the structure, function, and biodiversity of an

ecosystem. For example, ecologically relevant end points might contribute to the food base (e.g., primary production), provide habitat, promote regeneration of critical resources (e.g., nutrient cycling), or reflect the structure of the community, ecosystem, or landscape (e.g., species diversity). Ecological relevance becomes most useful when it is possible to identify the potential cascade of adverse effects that could result from a critical initiating effect such as a change in ecosystem function. The selection of assessment end points that address both specific organisms of concern and landscape-level ecosystem processes becomes increasingly important (and more difficult) in landscape-level risk assessments. In these cases, it may be possible to select one or more species and an ecosystem process to represent larger functional community or ecosystem processes. Extrapolations like these must be explicitly described in the conceptual model (see Section 27.2.2).

Susceptibility to Stressors Ecological resources or entities are considered susceptible if they are sensitive to a human-induced stressor to which they are exposed. *Sensitivity* represents how readily an ecological entity responds to a particular stressor. Measures of sensitivity may include mortality or decreased growth or fecundity resulting from exposure to a toxicant, or behavioral abnormalities such as avoidance of food source areas or nesting sites because of the proximity of stressors such as noise or habitat alteration. Sensitivity is directly related to the mode of action of the stressors. For example, chemical sensitivity is influenced by individual physiology, genetics, and metabolism. Sensitivity also is influenced by individual and community life history characteristics. For example, species with long life cycles and low reproductive rates will be more vulnerable to extinction from increases in mortality than those with short life cycles and high reproductive rates. Species with large home ranges may be more sensitive to habitat fragmentation compared with those species with smaller home ranges within a fragment. Sensitivity may be related to the life stage of an organism when exposed to a stressor. Young animals often are more sensitive to stressors than adults. In addition, events like migration and molting often increase sensitivity because they require significant energy expenditure, which makes these organisms more vulnerable to stressors. Sensitivity also may be increased by the presence of other stressors or natural disturbances.

Exposure is the other key determinant in susceptibility. In ecological terms, exposure can mean co-occurrence, contact, or the absence of contact, depending on the stressor and assessment end point. The characteristics and conditions of exposure will influence how an ecological entity will respond to a stressor and thus will determine what ecological entities might be susceptible. Therefore, one must consider information on the proximity of an ecological entity to the stressor along with the timing (e.g., frequency and duration relative to sensitive life stages) and intensity of exposure. Note that adverse effects may be observed even at very low stressor exposures if a necessary resource is limited during a critical life stage. For example, if fish are unable to find suitable nesting sites during their reproductive phase, risk is significant even when water quality is high and food sources are abundant.

Exposure may take place at one point in space and time, but effects may not arise until another place or time. Both life history characteristics and the circumstances of exposure influence susceptibility in this case. For example, exposure

of a population to endocrine-modulating chemicals can affect the sex ratio of the offspring, but the population impacts of this exposure may not become apparent until years later when the cohort of affected animals begins to reproduce. Delayed effects and multiple stressor exposures add complexity to evaluations of susceptibility. For example, although toxicity tests may determine receptor sensitivity to one stressor, the degree of susceptibility may depend on the co-occurrence of another stressor that significantly alters receptor response. Again, conceptual models need to reflect these additional factors.

Defining Assessment End Points Assessment end points provide a transition between management goals and the specific measures used in an assessment by helping to identify measurable attributes to quantify and model. However, in contrast to management goals, no intrinsic value is assigned to the end point so it does not contain words such as *protect* or *maintain*, and it does not indicate a desirable direction for change. Two aspects are required to define an assessment end point. The first is the valued ecological entity such as a species, a functional group of species, an ecosystem function or characteristic, or a specific valued habitat. The second is the characteristic about the entity of concern that is important to protect and is potentially at risk.

Expert judgment and an understanding of the characteristics and function of an ecosystem are important for translating general goals into usable assessment end points. End points that are too broad and vague (ecological health) cannot be linked to specific measurements. End points that are too narrowly defined (hatching success of bald eagles) may overlook important characteristics of the ecosystem and may fail to include critical variables. Clearly defined assessment end points provide both direction and boundaries for the risk assessment.

Assessment end points directly influence the type, characteristics, and interpretation of data and information used for analysis and the scale and character of the assessment. For example, an assessment end point such as "fecundity of bivalves" defines local population characteristics and requires very different types of data and ecosystem characterization compared to "aquatic community structure and function." When concerns are on a local scale, the assessment end points should not focus on landscape concerns. But if ecosystem processes and landscape patterns are being considered, survival of a single species would provide inadequate representation of this larger scale.

The presence of multiple stressors also influences the selection of assessment end points. When it is possible to select one assessment end point that is sensitive to many of the identified stressors yet responds in different ways to different stressors, it is possible to consider the combined effects of multiple stressors while still discriminating among effects. For example, if the recruitment of a fish population is the assessment end point, it is important to recognize that recruitment may be adversely affected at several life stages, in different habitats, through different ways, by different stressors. The measures of effect, exposure, and ecosystem and receptor characteristics chosen to evaluate recruitment provide a basis for discriminating among different stressors, individual effects, and their combined effect.

Although many potential assessment end points may be identified, practical considerations often drive their selection. For example, assessment end points

usually must reflect environmental values that are protected by law or that environmental managers and the general public recognize as a critical resource or an ecological function that would be significantly impaired if the resource were altered. Another example of a practical consideration is the extrapolation across scales of time, space, or level of biological organization. When the attributes of an assessment end point can be measured directly, extrapolation is unnecessary and this uncertainty is avoided. Assessment end points that cannot be linked with measurable attributes are not appropriate for a risk assessment. However, assessment end points that cannot be measured directly but can be represented by surrogate measures that are easily monitored and modeled can still provide a good foundation for the risk assessment.

27.2.2 Developing Conceptual Models

Conceptual models link anthropogenic activities with stressors and evaluate the relationships among exposure pathways, ecological effects, and ecological receptors. The models also may describe natural processes that influence these relationships. Conceptual models include a set of risk hypotheses that describe predicted relationships between stressor, exposure, and assessment end-point response, along with the rationale for their selection. Risk hypotheses are hypotheses in the broad scientific sense; they do not necessarily involve statistical testing of null and alternative hypotheses or any particular analytical approach. Risk hypotheses may predict the effects of a stressor or they may postulate what stressors may have caused observed ecological effects.

Diagrams can be used to illustrate the relationships described by the conceptual model and risk hypotheses. Conceptual model diagrams are useful tools for communicating important pathways and for identifying major sources of uncertainty. These diagrams and risk hypotheses can be used to identify the most important pathways and relationships to consider in the analysis phase. The hypotheses considered most likely to contribute to risk are identified for subsequent evaluation in the risk assessment.

The complexity of the conceptual model depends on the complexity of the problem, the number of stressors and assessment end points being considered, the nature of effects, and the characteristics of the ecosystem. For single stressors and single assessment end points, conceptual models can be relatively simple relationships. In cases where conceptual models describe both the pathways of individual stressors and assessment end points and the interaction of multiple and diverse stressors and assessment end points, several submodels would be required to describe individual pathways. Other models may then be used to explore how these individual pathways interact. An example of a conceptual model for a watershed is shown in Figure 27.2.

27.2.3 Selecting Measures

The last step in the problem formulation phase is the development of an analysis plan or proposal that identifies measures to evaluate each risk hypothesis and that describes the assessment design, data needs, assumptions, extrapolations, and specific methods for conducting the analysis. There are three categories of measures

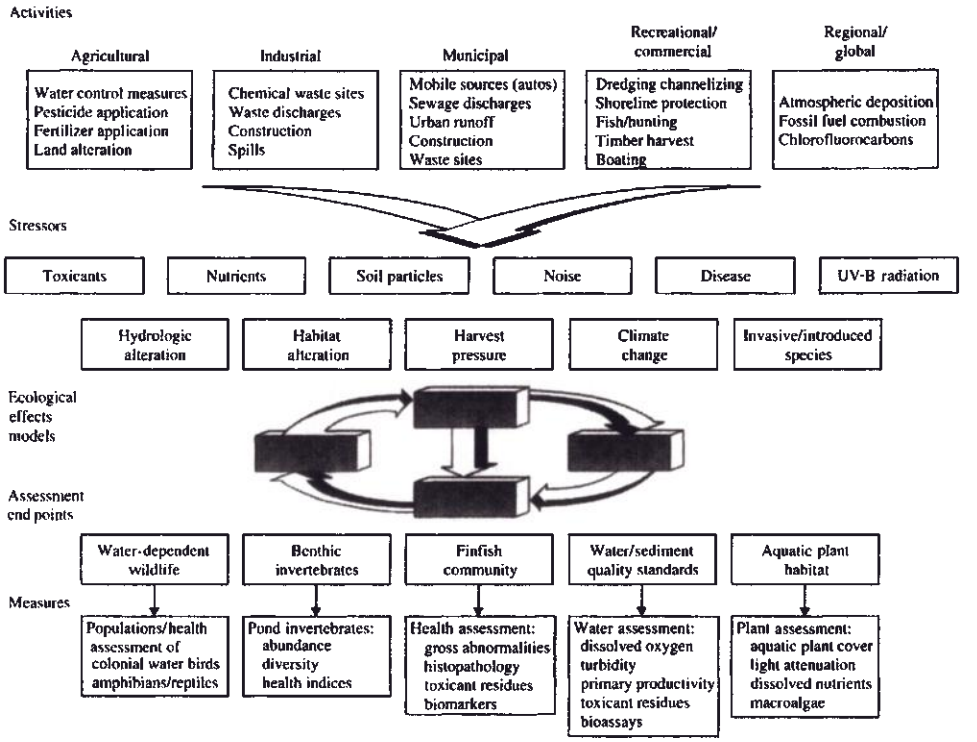

Figure 27.2 An example of a conceptual model for a watershed. Human activities, shown at the top of the diagram, result in various stressors that induce ecological effects. Assessment end points and related measures that are associated with these effects are shown at the bottom of the diagram.

that can be selected: (1) *measures of effect* (also called *measurement end points*) are measures used to evaluate the response of the assessment end point when exposed to a stressor; (2) *measures of exposure* are measures of how exposure may be occurring, including how a stressor moves through the environment and how it may co-occur with the assessment end point; and (3) *measures of ecosystem and receptor characteristics* include ecosystem characteristics that influence the behavior and location of assessment end points, the distribution of a stressor, and the life history characteristics of the assessment end point that may affect exposure or response to the stressor. These diverse measures increase in importance as the complexity of the assessment increases.

An important consideration in the identification of these measures is their response sensitivity and ecosystem relevance. Response sensitivity is usually highest with measures at the lower levels of biological organization, but the ecosystem relevance is highest at the higher levels of biological organization. This dichotomy is illustrated in Figure 27.3. In general, the time required to illicit a response also increases with the level of biological organization. Note that toxicologists focus on measures at lower levels of biological organization, relying upon an extrapolation based on the tenet that effects of toxicants on populations and communities are

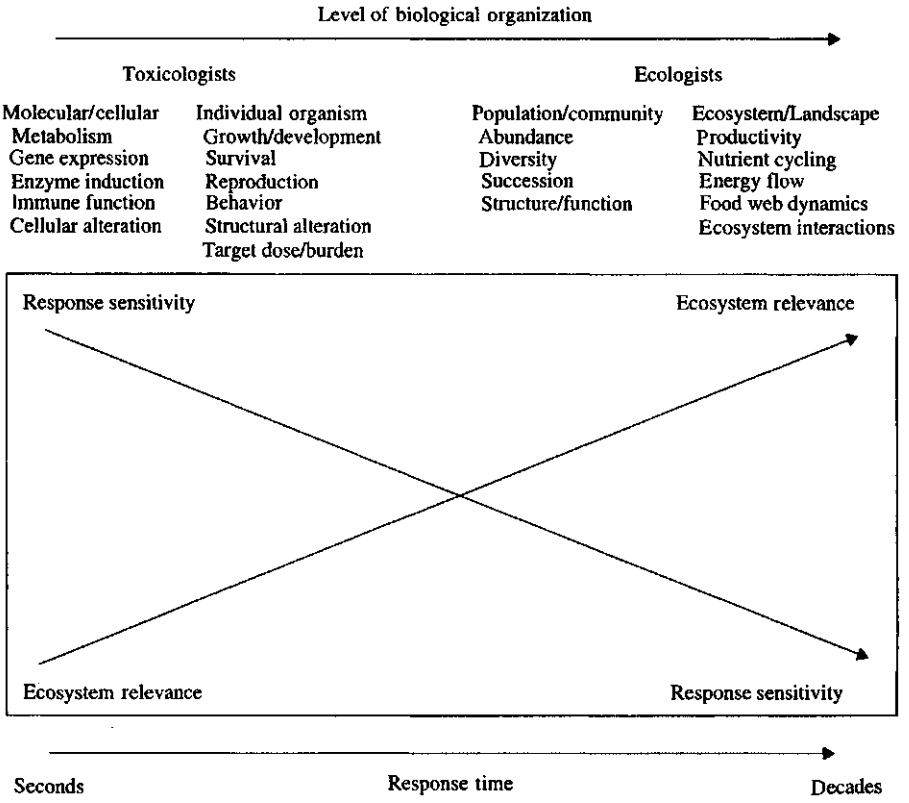

Figure 27.3 The response time and sensitivity of an ecological receptor is a function of the level of biological organization. Higher levels of organization have greater ecosystem relevance. However, as the level of biological organization increases, response time increases, sensitivity decreases, and causal relationships become more uncertain. Ecological risk assessments must balance the need for sensitive, timely, and well-established responses with ecological relevance.

initiated at the molecular/cellular level, and if this insult is not corrected for, or adapted to, then effects on physiological systems and individual organisms will result. For certain toxic modes of action (e.g., reproductive toxicity), this could result in effects at the population and community levels. In contrast, ecologists focus on measures at the population level or higher for obvious reasons of ecological relevance. A combination of measures is often necessary to provide reasonable sensitivity, ecosystem relevance, and causal relationships.

27.3 ANALYZING EXPOSURE AND EFFECTS INFORMATION

The second phase of ecological risk assessment, the analysis phase, includes two principal activities: characterization of exposure and characterization of ecological effects (Figure 27.1).

27.3.1 Characterizing Exposure

In exposure characterization, credible and relevant data are analyzed to describe the source(s) of stressors, the distribution of stressors in the environment, and the contact or co-occurrence of stressors with ecological receptors. An exposure profile is developed that identifies receptors and exposure pathways, describes the intensity and spatial and temporal extent of exposure, describes the impact of variability and uncertainty on exposure estimates, and presents a conclusion about the likelihood that exposure will occur.

A source description identifies where the stressor originates, describes what stressors are generated, and considers other sources of the stressor. Exposure analysis may start with the source when it is known, but some analyses may begin with known exposures and attempt to link them to sources, while other analyses may start with known stressors and attempt to identify sources and to quantify contact or co-occurrence. The source description includes what is known about the intensity, timing, and location of the stressor and whether other constituents emitted by the source influence transport, transformation, or bioavailability of the stressor of interest.

Many stressors have natural counterparts and/or multiple sources that must be considered. For example, many chemicals occur naturally (e.g., most metals), are generally widespread due to multiple sources (e.g., polycyclic aromatic hydrocarbons), or may have significant sources outside the boundaries of the current assessment (e.g., regional atmospheric deposition of polychlorinated biphenyl (PCBs)). Many physical stressors also have natural counterparts such as sedimentation from construction activities versus natural erosion. In addition, human activities may change the magnitude or frequency of natural disturbance cycles such as the frequency and severity of flooding. Source characterization can be particularly important for new biological stressors (e.g., invasive species) since many of the strategies for reducing risks focus on preventing entry in the first place. Once the source is identified, the likelihood of entry may be characterized qualitatively.

Because exposure occurs where receptors co-occur with or contact stressors in the environment, characterizing the spatial and temporal distribution of a stressor is a necessary precursor to estimating exposure. The stressor's spatial and temporal distribution in the environment is described by evaluating the pathways that stressors take from the source as well as the formation and subsequent distribution of secondary stressors. For chemical stressors, the evaluation of pathways usually follows the type of transport and fate modeling described in Chapter 26. Some physical stressors such as sedimentation also can be modeled, but other physical stressors require no modeling because they eliminate entire ecosystems or portions of them, such as when a wetland is filled, a resource is harvested, or an area is flooded.

The movement of biological stressors has been described as diffusion and/or jump–dispersal processes. Diffusion involves a gradual spread from the site of introduction and is a function primarily of reproductive rates and motility. Jump–dispersal involves erratic spreads over periods of time, usually by means of a vector. The gypsy moth and zebra mussel have spread this way: the gypsy moth via egg masses on vehicles and the zebra mussel via boat ballast water. Biological stressors can use both diffusion and jump–dispersal strategies, which makes it difficult to

predict dispersal rates. An additional complication is that biological stressors are influenced by their own survival and reproduction.

The creation of secondary stressors can greatly alter risk. Secondary stressors can be formed through biotic or abiotic transformation processes and may be of greater or lesser concern than the primary stressor. Physical disturbances can generate secondary stressors, such as when the removal of riparian vegetation results in increased nutrients, sedimentation, and altered stream flow. For chemicals, the evaluation of secondary stressors usually focuses on metabolites or degradation products. In addition, secondary stressors can be formed through ecosystem processes. For example, nutrient inputs into an estuary can decrease dissolved oxygen concentrations because they increase primary production and subsequent decomposition. A changeover from an aerobic to an anaerobic environment often is accompanied by the production of sulfide via sulfate-reducing bacteria. Sulfide can act as a secondary stressor to oxygen-dependent organisms, but it also can reduce exposure to metals through the precipitation of metal sulfides (see Chapter 26).

The distribution of stressors in the environment can be described using measurements, models, or a combination of the two. If stressors have already been released, direct measurements of environmental media or a combination of modeling and measurement is preferred. However, a modeling approach may be necessary if the assessment is intended to predict future scenarios or if measurements are not possible or practicable.

27.3.2 Characterizing Ecological Effects

In ecological effect characterization, relevant data are analyzed to evaluate stressor–response relationships and/or to provide evidence that exposure to a stressor causes an observed response. The characterization describes the effects that are elicited by a stressor, links these effects with the assessment end points, and evaluates how the effects change with varying stressor levels. The conclusions of the ecological effect characterization are summarized in a stressor–response profile.

Analyzing Ecological Response Ecological response analysis has three primary components: determining the relationship between stressor exposure and ecological effects, evaluating the plausibility that effects may occur or are occurring as a result of the exposure, and linking measurable ecological effects with the assessment end points.

Evaluating ecological risks requires an understanding of the relationships between stressor exposure and resulting ecological responses. The stressor–response relationships used in a particular assessment depend on the scope and nature of the ecological risk assessment as defined in problem formulation and as reflected in the analysis plan. For example, a point estimate of an effect (such as an median lethal concentration (LC50)) might be compared with point estimates from other stressors. The stressor–response function (e.g., shape of the curve) may be critical for determining the presence or absence of an effect threshold or for evaluating incremental risks, or stressor–response functions may be used as input for ecological effects models. If sufficient data are available, cumulative distribution functions can be constructed using multiple point estimates of effects. Process models that already incorporate empirically derived stressor–response functions can also be used.

However, many stressor–response relationships are very complex and ecological systems frequently show responses to stressors that involve abrupt shifts to new community or system types.

In simple cases, the response will be one variable (e.g., mortality) and quantitative univariate analysis can be used. If the response of interest is composed of many individual variables (e.g., species abundances in an aquatic community), multivariate statistical techniques must be used. Multivariate techniques (e.g., factor and cluster analysis) have a long history of use in ecology but have not yet been extensively applied in risk assessment. Stressor–response relationships can be described using any of the dimensions of exposure (i.e., intensity, time, or space). Intensity is probably the most familiar dimension and is often used for chemicals (e.g., dose, concentration). The duration of exposure can also be used for chemical stressor–response relationships; for example, median acute effect levels are always associated with a time parameter (e.g., 24, 48, and 96 h). Both the time and spatial dimensions of exposure can be important for physical disturbances such as flooding. Single-point estimates and stressor–response curves can be generated for some biological stressors. For pathogens such as bacteria and fungi, inoculum levels may be related to the level of symptoms in a host or to the actual signs of the pathogen. For other biological stressors such as introduced species, developing simple stressor–response relationships may be inappropriate.

Causality is the relationship between cause (one or more stressors) and effect (assessment end-point response to one or more stressors). Without a sound basis for linking cause and effect, uncertainty in the conclusions of an ecological risk assessment will be high. Developing causal relationships is especially important for risk assessments driven by observed adverse ecological effects such as fish kills or long-term declines in a population. Criteria need to be established for evaluating causality. For chemicals, ecotoxicologists have slightly modified Koch's postulates to provide evidence of causality:

1. The injury, dysfunction, or other putative effect of the toxicant must be regularly associated with exposure to the toxicant and with any contributory causal factors.
2. Indicators of exposure to the toxicant must be found in the affected organisms.
3. The toxic effects must be seen when normal organisms or communities are exposed to the toxicant under controlled conditions, and any contributory factors should be manifested in the same way during controlled exposures.
4. The same indicators of exposure and effects must be identified in the controlled exposures as in the field.

While useful as an ideal, this approach may not be practical if resources for experimentation are not available or if an adverse effect may be occurring over such a wide spatial extent that experimentation and correlation may prove difficult or may yield equivocal results. In most cases, extrapolation will be necessary to evaluate causality. The scope of the risk assessment also influences extrapolation through the nature of the assessment end point. Preliminary assessments that evaluate risks to general trophic levels, such as fish and birds, may extrapolate among different genera or families to obtain a range of sensitivity to the stressor. On the other hand,

assessments concerned with management strategies for a particular species may employ population models.

Whatever methods are employed to link assessment end points with measures of effect, it is important to apply the methods in a manner consistent with sound ecological and toxicological principles. For example, it is inappropriate to use structure–activity relationships to predict toxicity from chemical structure unless the chemical under consideration has a similar mode of toxic action to the reference chemicals. Similarly, extrapolations from upland avian species to waterfowl may be more credible if factors such as differences in food preferences, physiology, and seasonal behavior (e.g., mating and migration habits) are considered.

Finally, many extrapolation methods are limited by the availability of suitable databases. Although these databases are generally largest for chemical stressors and aquatic species, even in these cases, data do not exist for all taxa or effects. Chemical effects databases for mammals, amphibians, or reptiles are extremely limited, and there is even less information on most biological and physical stressors. Extrapolations and models are only as useful as the data on which they are based and should recognize the great uncertainties associated with extrapolations that lack an adequate empirical or process-based rationale.

Developing a Stressor–Response Profile The final activity of the ecological response analysis is developing a stressor–response profile to evaluate single species, populations, general trophic levels, communities, ecosystems, or landscapes—whatever is appropriate for the defined assessment end points. For example, if a single species is affected, effects should represent appropriate parameters such as effects on mortality, growth, and reproduction, while at the community level, effects may be summarized in terms of structure or function depending on the assessment end point. At the landscape level, there may be a suite of assessment end points and each should be addressed separately. The stressor–response profile summarizes the nature and intensity of effect(s), the timescale for recovery (where appropriate), causal information linking the stressor with observed effects, and uncertainties associated with the analysis.

27.4 CHARACTERIZING RISK

Risk characterization is the final phase of an ecological risk assessment (Figure 27.1). During risk characterization, risks are estimated and interpreted, and the strengths, limitations, assumptions, and major uncertainties are summarized. Risks are estimated by integrating exposure and stressor–response profiles using a wide range of techniques such as comparisons of point estimates or distributions of exposure and effects data, process models, or empirical approaches such as field observational data. Risks are described by evaluating the evidence supporting or by refuting the risk estimate(s) and interpreting the adverse effects on the assessment end point. Criteria for evaluating adversity include the nature and intensity of effects, spatial and temporal scales, and the potential for recovery. Agreement among different lines of evidence of risk increases confidence in the conclusions of a risk assessment.

27.4.1 Estimating Risk

Risk estimation determines the likelihood of adverse effects to assessment end points by integrating exposure and effects data and by evaluating any associated uncertainties. The process uses the exposure and stressor–response profiles. Risks can be estimated by one or more of the following approaches: (1) estimates based on best professional judgment and expressed as qualitative categories such as low, medium, or high; (2) estimates comparing single-point estimates of exposure and effects such as a simple ratio of exposure concentration to effects concentration (quotient method); (3) estimates incorporating the entire stressor–response relationship often as a nonlinear function of exposure; (4) estimates incorporating variability in exposure and effects estimates providing the capability to predict changes in the magnitude and likelihood of effects at different exposure scenarios; (5) estimates based on process models that rely partially or entirely on theoretical approximations of exposure and effects; and (6) estimates based on empirical approaches, including field observational data. An example of the first approach, using qualitative categorization, is shown in Figure 27.4.

27.4.2 Describing Risk

After risks have been estimated, available information must be integrated and interpreted to form conclusions about risks to the assessment end points. Risk descriptions include an evaluation of the lines of evidence supporting or refuting the risk estimate(s) and an interpretation of the adverse effects on the assessment end point. Confidence in the conclusions of a risk assessment may be increased by using several lines of evidence to interpret and compare risk estimates. These lines of evidence may be derived from different sources or by different techniques relevant to adverse effects on the assessment end points, such as quotient estimates, modeling results, field experiments, or field observations. Some of the factors to consider when evaluating separate lines of evidence are

- the relevance of evidence to the assessment end points,
- the relevance of evidence to the conceptual model,
- the sufficiency and quality of data and experimental designs used in supporting studies,
- the strength of cause/effect relationships, and
- the relative uncertainties of each line of evidence and their direction.

At this point in risk characterization, the changes expected in the assessment end points have been estimated and described. The next step is to interpret whether these changes are considered adverse and meaningful. Meaningful adverse changes are defined by ecological and/or social concerns and, thus, usually depend on the best professional judgment of the risk assessor. Five criteria have been proposed by EPA for evaluating adverse changes in assessment end points:

1. Nature of effects
2. Intensity of effects

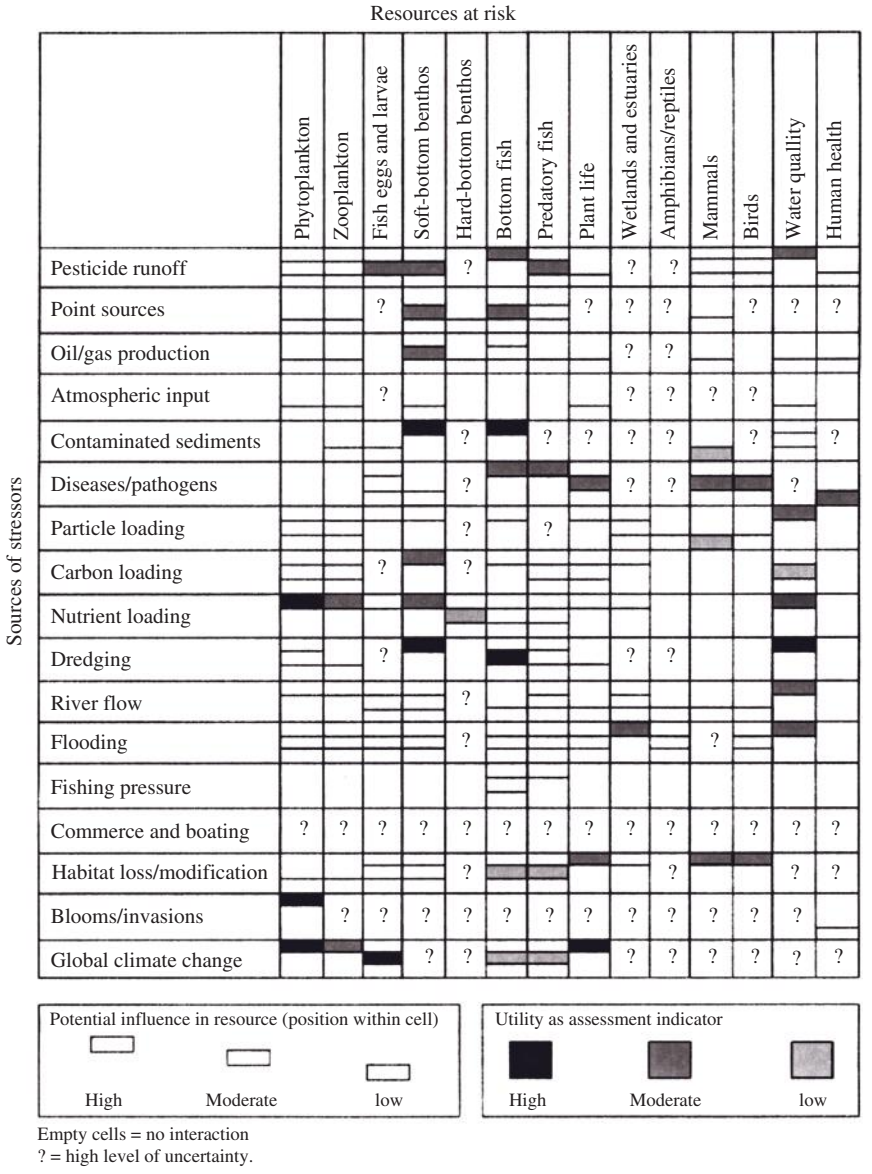

Figure 27.4 An example of a qualitative categorization of an ecological risk for a hypothetical matrix of stressors and resources at risk.

3. Spatial scale
4. Temporal scale
5. Potential for recovery

The extent to which the five criteria are evaluated depends on the scope and complexity of the ecological risk assessment. However, understanding the underlying

assumptions and science policy judgments is important even in simple cases. For example, when exceedance of a previously established decision rule such as a benchmark stressor level or water quality criteria is used as evidence of adversity, the reasons why exceedances of the benchmark are considered adverse should be clearly understood.

To distinguish ecological changes that are adverse from those ecological events that are within the normal pattern of ecosystem variability or result in little or no meaningful alteration of biota, it is important to consider the nature and intensity of effects. For example, an assessment end point involving survival, growth, and reproduction of a species must consider whether predicted effects involve survival and reproduction or only growth, or if the survival of the offspring is affected, the relative loss must be considered.

It is important to consider both the ecological and statistical contexts of an effect when evaluating intensity. For example, a statistically significant 1% decrease in fish growth may not be relevant to an assessment end point of fish population viability, and a 10% decline in reproduction may be worse for a population of slowly reproducing marine mammals than for rapidly reproducing planktonic algae.

Natural ecosystem variation can make it very difficult to observe (detect) stressor-related perturbations. For example, natural fluctuations in marine fish populations are often very large and cyclic events (e.g., fish migration) are very important in natural systems. Predicting the effects of anthropogenic stressors against this background of variation can be very difficult. Thus, a lack of statistically significant effects in a field study does not automatically mean that adverse ecological effects are absent. Rather, factors such as statistical power to detect differences, natural variability, and other lines of evidence must be considered in reaching conclusions about risk.

Spatial and temporal scales also need to be considered in assessing the adversity of the effects. The spatial dimension encompasses both the extent and pattern of effect as well as the context of the effect within the landscape. Factors to consider include the absolute area affected, the extent of critical habitats affected compared to a larger area of interest, and the role or use of the affected area within the landscape. Adverse effects to assessment end points vary with the absolute area of the effect. A larger affected area may be (1) subject to a greater number of other stressors, increasing the complications from stressor interactions; (2) more likely to contain sensitive species or habitats; or (3) more susceptible to landscape-level changes because many ecosystems may be altered by the stressors.

Nevertheless, a smaller area of effect is not always associated with lower risk. The function of an area within the landscape may be more important than the absolute area. Destruction of small but unique areas, such as submerged vegetation at the land–water margin, may have important effects on local wildlife populations. Also, in river systems, both riffle and pool areas provide important microhabitats that maintain the structure and function of the total river ecosystem. Stressors acting on some of these microhabitats may present a significant risk to the entire system. Spatial factors also are important for many species because of the linkages between ecological landscapes and population dynamics. Linkages between one or more landscapes can provide refuge for affected populations, and

species may require adequate corridors between habitat patches for successful migration.

The temporal scale for ecosystems can vary from seconds (photosynthesis, prokaryotic reproduction) to centuries (global climate change). Changes within a forest ecosystem can occur gradually over decades or centuries and may be affected by slowly changing external factors such as climate. The timescale of stressor-induced changes operates within the context of multiple natural timescales. In addition, temporal responses for ecosystems may involve intrinsic time lags so that responses from a stressor may be delayed. Thus, it is important to distinguish the long-term impacts of a stressor from the immediately visible effects. For example, visible changes resulting from eutrophication of aquatic systems (turbidity, excessive macrophyte growth, population decline) may not become evident for many years after initial increases in nutrient levels.

Considering the temporal scale of adverse effects leads us to a consideration of recovery. Recovery is the rate and extent of return of a population or community to a condition that existed before the introduction of a stressor. Because ecosystems are dynamic and even under natural conditions are constantly changing in response to changes in the physical environment (weather, natural catastrophes, etc.) or other factors, it is unrealistic to expect that a system will remain static at some level or will return to exactly the same state that it was before it was disturbed. Thus, the attributes of a "recovered" system must be carefully defined. Examples might include productivity declines in a eutrophic system, reestablishment of a species at a particular density, species recolonization of a damaged habitat, or the restoration of the health of diseased organisms.

Recovery can be evaluated in spite of the difficulty in predicting events in ecological systems. For example, it is possible to distinguish changes that are usually reversible (e.g., recovery of a stream from sewage effluent discharge), frequently irreversible (e.g., establishment of introduced species), and always irreversible (e.g., species extinction). It is important to consider whether significant structural or functional changes have occurred in a system that might render changes irreversible. For example, physical alterations such as deforestation can change soil structure and seed sources such that forests cannot easily grow again.

Natural disturbance patterns can be very important when evaluating the likelihood of recovery from anthropogenic stressors. Ecosystems that have been subjected to repeated natural disturbances may be more vulnerable to anthropogenic stressors (e.g., overfishing). Alternatively, if an ecosystem has become adapted to a disturbance pattern, it may be affected when the disturbance is removed (fire-maintained grasslands). The lack of natural analogues makes it difficult to predict recovery from novel anthropogenic stressors such as exposure to synthetic chemicals.

The relative rate of recovery also can be estimated. For example, fish populations in a stream are likely to recover much faster from exposure to a degradable chemical than from habitat alterations resulting from stream channelization. It is critical to use knowledge of factors such as the temporal scales of organisms' life histories, the availability of adequate stock for recruitment, and the interspecific and trophic dynamics of the populations in evaluating the relative rates of recovery. A fisheries stock or forest might recover in several

decades, a benthic infaunal community in years, and a planktonic community in weeks to months.

27.5 MANAGING RISK

When risk characterization is complete, a description of the risk assessment is communicated to the risk manager (Figure 27.1) to support a risk management decision. This communication usually is a report and might include a

- Description of risk assessor/risk manager planning results;
- Review of the conceptual model and the assessment end points;
- Discussion of the major data sources and analytical procedures used;
- Review of the stressor–response and exposure profiles;
- Description of risks to the assessment end points, including risk estimates and adversity evaluations;
- Summary of major areas of uncertainty and the approaches used to address them; and
- Discussion of science policy judgments or default assumptions used to bridge information gaps, and the basis for these assumptions.

After the risk assessment is completed, risk managers may consider whether additional follow-up activities are required. Depending on the importance of the assessment, confidence level in the assessment results, and available resources, it may be advisable to conduct another iteration of the risk assessment in order to facilitate a final management decision. Ecological risk assessments are frequently designed in sequential tiers that proceed from simple, relatively inexpensive evaluations to more costly and complex assessments. Initial tiers are based on conservative assumptions, such as maximum exposure and ecological sensitivity. When an early tier cannot sufficiently define risk to support a management decision, a higher assessment tier that may require either additional data or applying more refined analysis techniques to available data may be needed. Higher tiers provide more ecologically realistic assessments while making less conservative assumptions about exposure and effects.

Another option is to proceed with a management decision based on the risk assessment and to develop a monitoring plan to evaluate the results of the decision. For example, if the decision was to mitigate risks through exposure reduction, monitoring could help determine whether the desired reduction in exposure (and effects) was achieved. Monitoring is also critical for determining the extent and nature of any ecological recovery that may be occurring.

Ecological risk assessment is important for environmental decision making because of the high cost of eliminating environmental risks associated with human activities and the necessity of making regulatory decisions in the face of uncertainty. Ecological risk assessment provides only a portion of the information required to make risk management decisions, but this information is critical to scientifically defensible risk management. Thus, ecological risk assessments should

provide input to a diverse set of environmental decision-making processes, such as the regulation of hazardous waste sites, industrial chemicals, and pesticides, or the management of watersheds affected by multiple nonchemical and chemical stressors.

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SAMPLE QUESTIONS

1. Describe the process of assessing the risk of a stressor to an ecosystem.

2. Describe the difference and relationship between an assessment end point and a measurement end point. Give an example.

3. Describe one (or two) fundamental differences between human health risk assessment and ecological risk assessment.

4. Describe the general relationship among the response time, sensitivity, level of biological organization, and ecological relevance of measurements that can be used to assess the response of an ecological receptor to a stressor.