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CHAPTER 29

ECOTOXICOLOGY

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INTRODUCTION TO ECOTOXICOLOGY

The field of environmental toxicology, particularly as related to the area of ecotoxicology, continues to be a rapidly developing discipline of environmental science (Connell and Miller, 1984; Duffus, 1980; Guthie and Perry, 1980; Hoffman et al., 1995; Moriarity, 1988; Truhaut, 1977). The term *ecotoxicology* was introduced by Truhaut in 1969 (Truhaut, 1977) and this field is a natural extension of toxicology. It is best defined as the study of the fate and effects of toxic substances on an ecosystem and is based on scientific research employing both field and laboratory methods (Kendall, 1982; Kendall, 1992; and Hoffman et al., 1995). Environmental toxicology as it is related to ecotoxicology requires an

understanding of ecologic principles and theory as well as a grasp of how chemicals can affect individuals, populations, communities, and ecosystems (Kendall and Lacher, 1994; Hoffman et al., 1995). Measurements of biological impact are accomplished using either species-specific responses to toxicants (Smith, 1987) or impacts on higher levels of organization from individuals to populations, and so on. Ecotoxicology builds on the science of toxicology and the principles of toxicologic testing, though its emphasis is more at the population, community, and ecosystem levels (Moriarty, 1988). The ability to measure chemical transport and fate and exposure of organisms in ecotoxicologic testing is critical to the ultimate development of an ecologic risk assessment (U.S. EPA, 1992 a,b,c; Suter, 1993; Maughan, 1993).

Descriptions of ecotoxicologic methods and procedures have been offered by Cairns (1978) and Cairns et al. (1980) and more recently by Hoffman et al. (1995). Unlike standard toxicologic tests, which seek to define the cause-effect relationship with certain concentrations of toxicant exposure at a sensitive receptor site, ecotoxicologic testing attempts to evaluate cause and effects at higher levels of organization, but particularly on populations (National Academy of Sciences, 1975; Hoffman et al., 1995). To a large extent, the early tests (such as evaluating the effects of pesticides in fish and wildlife populations) generally employed species-specific tests in the laboratory (Smith, 1987). Tests of species included aquatic species such as *Daphnia magna,* fathead minnows (*Pimephales promelas*), the mosquitofish (*Gambusia affinis*); and, among wildlife, the northern bobwhite (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*) (Lamb and Kenaga, 1981). Arguments have continued over the last decade concerning the relevance of these few organisms to the larger ecosystem at risk. Methods for laboratory bioassays to measure the impact of chemical and nonchemical stressors on aquatic and terrestrial plants and animals continue to evolve. In addition, extrapolation of the results of these assays to field conditions and their utility in an ecologic risk assessment are active areas of research. To an even larger degree, the interrelationship or signals of animal sentinels responding to environmental toxicants as related to human health is an area of increasing interest and under rapid development (Kendall et al., 1998).

A critical component in ecotoxicologic testing is the integration of laboratory and field research (Kendall and Akerman, 1992). Laboratory toxicity bioassays define toxicant impact on individual organisms and on their biochemistry and physiology. Knowledge acquired in the laboratory is integrated with what is occurring under field conditions and is critical to understanding the complex set of parameters with which an organism must deal in order to reproduce or survive under toxicant exposures. Laboratory testing often limits the complexity of stress parameters except perhaps for isolating the toxicant. It is therefore difficult to interpret potential ecotoxicologic effects resulting from laboratory studies without data from pertinent field investigations. For these reasons, integrating laboratory and field research ensures that ecotoxicologic testing methods produce relevant data (Kendall and Lacher, 1994). Demands on ecotoxicologic testing methodologies will continue to increase as concern for environmental protection and chemical impacts increases. Scientific journals continue to publish increasing numbers of manuscripts on ecotoxicologic studies. Furthermore, there is an increasing interest in the relationship of the environment and potential environment toxicant stressors in human health implications. Therefore, this chapter, in addition to outlining some test methodologies for evaluating the effects of toxicants on invertebrates, vertebrates, and plants in aquatic and terrestrial ecosystems, also addresses the relationship of these endpoints to potential human health implications. The complexity and testing strategy in the aquatic versus terrestrial environment can be quite different, and this is one of the challenges currently faced by ecotoxicologic research. For this reason the chapter addresses both aquatic and terrestrial ecotoxicology to reflect the often different parameters involved in evaluating chemical impacts on aquatic versus terrestrial habitats. In recent years, the creation of major new environmental legislation—including the Food Quality Protection Act of 1996 and amendments to the Safe Drinking Water Act—has dictated a renewed evaluation of the relationship between environmental toxicants and potential impacts on the environment as related to human health implications. For this reason, the current chapter addresses some questions and issues related to the integration of environmental signals for toxicant stress and human health implications; in addition, discussion of ecologic risk assessment as related to applications of environmental toxicology data are expanded upon. Those reading this chapter should be aware that the increasing interest on sublethal impacts of contaminants on the environment, including those of biological and nonbiological origin, is being dealt with to a large degree by new environmental laws and by new strategies in risk assessment. These new strategies in risk assessment include probabilistic approaches and increased emphasis on relating environmental information to human health. These issues are addressed in the present chapter.

CHEMICAL MOVEMENT, FATE, AND EXPOSURE

To characterize chemical behavior, it is necessary to measure the chemical in different environmental compartments (e.g., air, soil, water, and biological systems), understand the movement and transport of the chemical within and among these compartments, and follow the chemical as it is metabolized, degraded, stored, or concentrated within each compartment. During the past half-century, intensive effort has been directed toward developing analytic techniques to detect and quantify minute concentrations of chemicals in environmental matrices (Murray, 1993; Blaser et al., 1995). One need only look at the myriad of studies investigating parts per quadrillion (ppq) concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to realize that environmental analytic chemistry has progressed substantially to complement the ever-increasing sensitivity of measurable toxicologic endpoints. Consider, for illustrative purposes, the fact that 1 ppq is 1 billion times smaller than a part per million (ppm), equating to approximately 1 g of salt in a billion metric tons of sugar. Nevertheless, it is well documented that environmental concentrations below 1 ppm of certain chemicals can have deleterious effects on different components of the ecosystem.

Chemodynamics

Chemical transport occurs both within environmental compartments (intraphase) and between them (interphase) (Thibodeaux, 1996; Mackay, 1991) and is critical to understanding and interpreting environmental toxicology data. A likely scenario for a chemical released into the environment entails its release into one environmental compartment; it is subsequently partitioned among environmental compartments; it is involved in movement and reactions within each compartment; it is partitioned between each compartment and the biota that reside in that compartment; and it finally reaches an active site in an organism at a high enough concentration for long enough to induce an effect. Chemodynamics is, in essence, the study of chemical release, distribution, degradation, and fate in the environment.

Contaminant transport through the environment is often predicted assuming thermodynamic equilibrium. While this assumption often does not hold, the approach is relatively straightforward and easy to apply. Although intraphase chemical transport is most easily approximated assuming thermodynamic equilibrium, better accuracy is possible using a steady-state model (Mackay, 1991). Abiotic and biotic reactions, which occur within a phase, result in significant changes in the physical and chemical properties of the compound, such as the oxidation state, lipophilicity, and volatility.

Combining these approaches facilitates prediction of the chemical concentration within the immediate vicinity of a particular organism. Chemodynamics can also describe chemical movement or absorption into organisms. Detoxification mechanisms, such as partitioning into adipose tissue, metabolism, and accelerated excretion, can significantly reduce, eliminate, or in some cases increase the toxic action of the chemical. Thus, an appreciation of chemodynamics aids in the prediction of chemical concentrations in compartments and serves as a resource for designing toxicologic experiments using the appropriate concentrations and forms of the chemical in question.

Single-Phase Chemical Behavior

Once a synthetic chemical enters the environment, it is acted upon primarily by natural forces. Models are used to predict the effect of natural forces on the movement of chemicals in the environment. This requires the incorporation of abiotic variables into valid models. These variables include temperature, wind and water-flow directions and velocities, incident solar radiation, atmospheric pressure and humidity, and the concentration of the chemical in one of four matrices: atmosphere (air), hydrosphere (water), lithosphere (soil), and biosphere (living organisms). Intraphase movement consists of mass transfer, diffusion, or dispersion within a single phase (Atkins, 1982). Concentration gradients result in movement within the medium. Contaminant persistence is a function of the stability of that chemical in a phase and its transport within that phase. Stability is a function of the physicochemical properties of a particular chemical and the kinetics of its degradation in the phase; these vary widely in and between classes of chemicals (Howard et al., 1991). Stability issues are difficult to predict and are often better handled by observation rather than modeling. Transport of chemicals in the environment, in contrast, is more predictable and is discussed in detail below.

Air The primary routes of contaminant entry into the atmosphere are through evaporation, stack emissions, and other matrices. Contaminant transport in air generally occurs much more rapidly than in the hydrosphere, as air has lower viscosity. Contaminant transport in air occurs primarily by diffusional processes or advection. Diffusion dominates in the very thin boundary layer between air and the other phases, the thickness of which is less than that of equivalent water-phase interfaces. The diffusion rate for a contaminant in air is approximately 100-fold faster than for the same contaminant in water and is a function of phase viscosity and existing concentration gradients. The contaminant diffusivity in air depends on its molecular weight compared to air, air temperature, the molecular separation at collision, the energy of molecular interaction, and Boltzmann's constant (Atkins, 1982). Wind currents transport airborne contaminants much more rapidly than does diffusion (Wark and Warner, 1981). Atmospheric stability affects the amount of turbulence and thus the degree of vertical mixing in the atmosphere. The stability of the atmosphere is considered neutral when the convective forces—heat transfer from warm ground surfaces and radiative cooling from the top of the cloud layer—are equal. Vertical mixing is at a maximum when heat transfer is greater than radiative cooling and at a minimum during inversion conditions. It is the latter condition that can trap higher concentrations of contaminants near the earth's surface.

Water Contaminants enter the hydrosphere by direct application, spills, wet and dry deposition, and interphase movement. In addition, chemicals enter the hydrosphere by direct dissolution of lighter-than-water spills in the form of slicks or from pools on the bottom of channels, rivers, or other waterways. Chemical movement in the hydrosphere occurs through diffusion, dispersion, and bulk flow of the water. In any flow, a stagnant boundary layer exists at the interface between phases or artificial boundaries. Overlying this layer is a section in which flow is laminar. Finally, above the laminar flow, the fluid is in turbulent flow. Contaminant movement in a mobile phase, in this case, water, is dominated by the turbulence of the mobile phase. If the water is stagnant, (e.g., in close proximity to a stationary phase such as soil or an artificial boundary), the chemical moves by molecular diffusion. As described for the other fluid environmental compartment, air, the diffusion rate depends on fixed characteristics such as the molecular weight of the contaminant (solute), the molecular weight of the water (solvent), water temperature, viscosity, and the association factor for water and dynamic characteristics such as the magnitude of the concentration gradient of the contaminant. These characteristics are referred to as the *diffusivity* of the contaminant-water mixture. Diffusional processes in water are several orders of magnitude faster than in soil.

Away from the boundaries of other media (i.e., air and soil), transport in water is dominated by turbulence. Even in seemingly still water, water is constantly moving in vertical and horizontal eddies. These eddies are small pockets of water that form and subside and, during the process, transport the contaminant. This mode of transport is defined as *eddy diffusion.* In addition, the contaminant can be rapidly transported by bulk flow (also referred to as *advection*) in the cases of streams and rivers. In advection, the rate of transport is proportional to stream velocity.

Soil Chemicals enter the lithosphere by processes similar to those for the hydrosphere. Soils have varying porosities due to their composition (percent sand, silt, clay, organic matter), but pores are invariably filled with either gas or fluids. Chemical movement in the soil occurs by diffusion in these fluids or by the movement of water through the voids between soil particles. Fluid-borne contaminants partition with the solid fraction of soil by processes closely resembling chromatography, in that chemical solubility in pore water, adsorption to soil particles, and pore-water velocity affect the rate of transport (Willard et al., 1988). The direction of diffusion will be from areas of high to areas of low concentration. The chemical diffusion rate in soil depends on molecular weight, soil temperature, the length of the path, and the magnitude of the concentration gradient (Shonnard et al., 1993), among other issues. Contaminants leave the soil by interphase transport or decomposition. Transformation of contaminants (as through microbial degradation) can be significant in soil due to the density and diversity of microorganisms in this compartment compared with water and air.

Chemical Transport between Phases

Once released, a chemical can enter any of the four matrices: the atmosphere by evaporation, the lithosphere by adsorption, the hy-

drosphere by dissolution, or the biosphere by absorption, inhalation, or ingestion (depending on the species). Once in a matrix, the contaminant can enter another matrix by interphase transport. Absorption by biota is considered under "Chemical Behavior and Bioavailability," below.

Air–Water A chemical can leave the water by volatilization. Conversely, an airborne contaminant can move into an aqueous phase by absorption. At equilibrium, the net rates of volatilization and absorption are equal and the total mass transfer of the contaminant is zero. In nonequilibrium conditions, the rate of net movement of a chemical from one phase to another depends on how far the system is away from equilibrium as well as the magnitude of the overall mass transfer coefficient (Mackay, 1991). In turn, this mass transfer coefficient depends on the physical properties of the solute (such as vapor pressure and solubility) and the magnitude of the bulk flow of both the air and the water. For example, ammonia desorbs most quickly from shallow, rapidly flowing streams with a brisk cross wind. Alternatively, the water–air interface (surface microlayer) can be a concentration point for materials, both natural and anthropogenic (Hardy, 1982; Gever et al., 1996).

Soil–Water A contaminant can leave the soil and enter the water through the process of desorption. Water-borne contaminants can also adsorb on soil particles. Again, the rate of mass transfer depends on the contaminant-specific overall mass transfer coefficient, the bulk flow velocity of the water over the water-soil interface, and physicochemical properties of the soil, such as particle size distribution and organic matter content. Partitioning of contaminants from water to soil or sediment is one of the key processes controlling exposure.

Soil–Air A contaminant may leave the soil and be transported into the overlying air through the process of volatilization. This process is dependent on the vapor pressure of the chemical and its affinity for the soil. Environmental processes that affect the thickness of the soil–air boundary layer (i.e., wind velocity) or contaminant sorption (i.e., soil moisture content), in turn, influence movement from soil to air. For example, more contaminant will be released from contaminated soil at higher wind velocities as well as from wet versus dry soil.

Chemical Behavior and Bioavailability

An appreciation of how physicochemical properties influence contaminant behavior is necessary to anticipate chemical concentrations and speciation in different environmental compartments. Such an appreciation is also valuable in developing an exposure characterization for the contaminant(s) of interest. Ultimately, the goal is to assess the potential bioconcentration (uptake of contaminants from the external environment), bioaccumulation (uptake of contaminants from the external environment and food), and biomagnification (increasing contaminant concentrations at higher trophic levels) in organisms. An investment in careful exposure characterization is worth the expense and effort.

In the environment, only a portion of the total quantity of chemical present is potentially available for uptake by organisms. This concept is referred to as the *biological availability* (or bioavailability) of a chemical. Chemical bioavailability in various environmental compartments ultimately dictates toxicity; therefore it is important to characterize exposure on a site-specific basis. For example, total mercury concentration in aquatic sediments does not necessarily correlate with mercury concentration in midge larvae of the genus *Chironomus*. Important considerations in the case of mercury include the mercury species (e.g., the oxidation state, whether organic or inorganic) as well as physical and chemical characteristics of the sediment matrix (e.g., acid volatile sulfide concentration, pH, pE) (Tinsley, 1979). To complicate matters, in most cases mercury will not exist as a single species but will be distributed among several stable forms. Hence, a simple analytic result of total mercury content does not sufficiently describe the hazard associated with the presence of the metal in sediment. The multiple influences of soil, sediment, and water quality on the bioavailability of environmental chemicals are important research areas.

Chemical bioavailability in the water column has been studied for years, yet many questions are still unanswered. The behavior of dissolved metals, for example, has been studied for over two decades. In the early seventies, much research concerned the influence of pH and water hardness on metal toxicity to algae and other aquatic organisms. This work led to the development of a model to predict metal toxicity based on pH and water hardness [U.S. Environmental Protection Agency (EPA), 1986a].

The behavior and bioavailability of contaminants in the water column have been shown to relate directly to their water solubility. However, the presence of certain constituents in water may affect the apparent water solubility of toxicants. Johnson-Logan and coworkers (1992) demonstrated the apparent solubility of the organochlorine insecticide chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a hexahydro-4,7-methano-1*H*-indene) to be enhanced almost 500 percent in groundwater containing 34 mg/L total organic carbon. This enhanced solubility resulted directly from partitioning of this hydrophobic insecticide into the dissolved organic carbon (DOC) fraction within the water column. The apparent increase in water solubility did not necessarily indicate an increase in pesticide bioavailability. Dissolved organic carbon may increase transport and mobility of organic contaminants in the water column but also reduce their bioavailability.

The behavior and bioavailability of sediment-incorporated xenobiotics is a complex phenomenon studied only recently. The awareness that many aquatic contaminants settle into sediments has prompted studies of metals and organics to characterize their fate and disposition within the complex sediment matrix. Deposition is a combination of physical, chemical, and biological processes that may ultimately change the form of the xenobiotic. Many metals are abiotically or biotically reduced as they are incorporated into sediments. Mercury is methylated through microbial reactions in the sediment. Methylmercury is typically more bioavailable and more toxic than inorganic mercury.

Characterization of processes that control metal bioavailability in sediments would facilitate the development of models to predict toxic threshold concentrations of metals in different sediments. Work with sediment-incorporated metals has emphasized divalent cations in anaerobic environments. Under these conditions, acid volatile sulfides (AVS) preferentially bind divalent cations. Initial work with AVS focused on cadmium (DiToro et al., 1990), which can react with the solid phase AVS to displace iron and form a cadmium sulfide precipitate:

$$Cd^{2+} + FeS(s) \leftrightarrow CdS(s) + Fe^{2+}$$

If the AVS quantity in sediment exceeds the quantity of added cadmium, the cadmium concentration in the interstitial water is not

detectable and the cadmium is not bioavailable, hence it is not toxic. This process can be extended to other cations including nickel, zinc, lead, copper, mercury, and perhaps chromium, arsenic, and silver (Ankley et al., 1991). Furthermore, there is thermodynamic evidence that the presence of one divalent cation, copper for example, may displace a previously bound divalent cation with weaker binding strength such as cadmium. This results in a greater concentration of bioavailable cadmium while sulfide-bound copper is less bioavailable. Thus, the bioavailable fraction of metals in sediments can be predicted by measuring AVS and the simultaneously extracted metals (SEM) that result during AVS extraction. If the molar ratio of SEM to AVS is <1, little or no toxicity should be expected; if the molar ratio of SEM to AVS is >1, the mortality of sensitive species can be expected (DiToro et al., 1992). This approach is not without controversy and, while many scientists believe that AVS plays a significant role in the bioavailability of divalent cations in anaerobic sediment, most would agree that AVS alone does not predict metal bioavailability. Other sediment factors including oxide and hydroxide layers undoubtedly play a role in metal bioavailability. In addition, the ability of sediment-dwelling organisms to oxidize their surrounding environment, thus breaking metal-sulfide bonds, should be further studied.

Organic chemicals residing in the sediment matrix undergo a variety of abiotic and biotic transformations. Predicting the intraphase movement of organics in sediments is extremely difficult, and in general, the processes that control such movement are poorly understood. For nonionic, nonmetabolized, nonpolar organics, however; equilibrium partitioning theory has been proposed as the basis for developing sediment quality criteria. This theory suggests that, in the sediment matrix, certain chemicals partition between interstitial water and the organic carbon fraction of the solids. At equilibrium, this partitioning can be predicted using laboratory-generated partitioning coefficients (e.g., K_{oc}). The resulting interstitial water concentration should induce the same exposure as a water-only exposure. Thus, the toxicity of chemicals in interstitial water can be predicted using the results of water column bioassays with the chemical. One assumption of this theory is that, for these chemicals, exposure of sediment-dwelling organisms occurs through interstitial water only and that chemicals partitioned onto solids are not bioavailable. A good review of this theory and supporting data can be found in the 1991 report of DiToro and colleagues.

In soils, sorption also controls the bioavailability of contaminants. An example of the importance of site-specific exposure characterization is highlighted by a series of experiments designed by researchers at the United States Environmental Protection Agency (U.S. EPA) (Weis et al., 1994). The finding that many forms of environmental lead are not well absorbed across the gastrointestinal tract disproved the assumption that all forms of lead in contaminated surface soil are equally hazardous (Table 29-1).

Table 29-1
Studies of Endocrine Disruption in Representative Wildlife Species

SPECIES	COMPOUND	EFFECTS
Invertebrates		
Molluscs	Tributyltin	Imposex
Insects	DDE	Metabolic masculinization
Fish		
White sucker (*Catostomus commersoni*)	Kraft mill effluent	Delayed maturation, induction of vitellogenin in male fish, reduced gonad size and development, altered sex steroid concentrations
Trout (*Salmo gairderi*)	Municipal sewer effluent (containing alkylphenols, and conjugated estrogens	Feminization of male fish, including induction of plasma vitellogenin
Amphibians		
African clawed frog (*Xenopus laevis*)	Estrogens	Sexual imprinting (100% females)
Reptiles		
Red-eared slider (*Trachemys scripta*)	Estrogens, pesticides	Sex reversal, gonadal aberrations, altered sex steroid
American alligator (*Alligator mississippiensis*)	Estrogens, pesticides	Sex reversal, gonadal and phallus malformations, altered sex steroid concentrations
Birds		
Herring gulls (*Larus argentatus*)	PCBs, pesticides	Masculinization, altered gonadal structure
American bald Eagle (*Haliaeetus leucocephalus*)	PCBs, dioxins, pesticides	Eggshell thinning, brain asymmetry
Mammals		
Beluga whale (*Delphinptenus leucas*)	Organochlorines, metals	Tumors, immune suppression, impaired reproduction
Mink (*Mustela vision*)	PCBs, dioxins	Impaired reproduction, fetal mortality

SOURCE: Kendall et al., 1998, with permission.

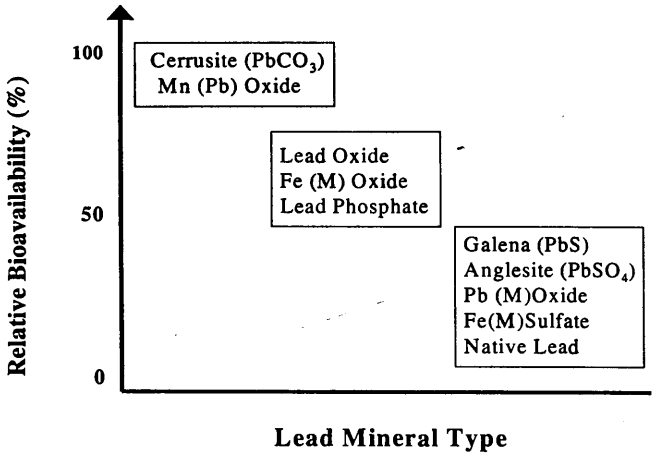

Figure 29-1. Gastrointestinal bioavailability of soil lead as a function of the physical and chemical nature of the exposure material (From Weis et al., 1994, with permission.)

Highly oxidized lead forms found in soils near mining and/or smelting sites is absorbed into blood nearly as well as freely soluble lead, while more reduced forms are only poorly absorbed.

Other lead forms were shown to be nearly as well absorbed as freely soluble lead acetate. Using an immature swine model as a surrogate (Weis and LaVelle, 1991) and a series of highly controlled animal studies, these investigators measured soil lead bioavailability ranging from less than 6 percent to greater than 90 percent relative to a soluble lead acetate reference substance (Fig. 29-1).

Tight sorption or sequestration of contaminants with increasing residence time in soil, often referred to as "aging," has also been documented (Pignatello et al., 1993; Hatzinger and Alexander, 1995), especially for lipophilic organic contaminants. Although the amount of contaminant in soil remains fairly constant, the fraction of the contaminant available to soil organisms is reported to significantly decrease with time. An important issue currently being addressed is the development of methods to assess the magnitude of available contaminant residues in soil (Kelsey and Alexander, 1997), including the use of sampling devices based on passive diffusion (Johnson et al., 1995; Awata et al., 1999).

BIOMARKERS

A fundamental challenge in environmental toxicology is relating the presence of a chemical in the environment with a valid prediction of ensuing hazard to potential biological receptors. Adverse health effects in biological receptors begin with exposure to a contaminant and can progress to damage or alteration in function of an organelle, cell, or tissue. Exposure of wildlife by contact to contaminated environmental media is defined as an *external dose,* whereas internalization of the contaminated media, via inhalation, ingestion, or dermal absorption, results in an *internal dose.* The amount of this internal dose necessary to elicit a response or health effect is referred to as the *biologically effective dose.*

Traditionally, environmental risk was assessed by chemical residue determination in samples of environmental media, combined with comparison to toxicity observed in species in contact with the media. This approach, although it yields useful information, has several limitations. The determination of chemical residues in environmental matrices is not simple and may require extensive sample cleanup leading to high per sample costs (U.S. EPA, 1986b). The availability of the chemicals in the environmental matrix to the biological receptor, or bioavailability, cannot be quantified by this approach. Depending upon the chemical, the environmental matrix, and the species, bioavailability may range from 100 percent to a fraction of a percent. To overcome this problem, chemical residue analysis of tissues containing the biological receptor may be performed [Agency for Toxic Substances and Disease Registry (ATSDR), 1994]. This approach, however, is often more difficult and expensive than the cost of the analysis of environmental matrices and yields no information on toxicologic response. In addition, the toxicokinetics and toxicodynamics of a contaminant in a particular species determines whether an exposure is capable of an adverse response. A biomarker-based approach resolves many of these difficulties by providing a direct measure of toxicant effects in the affected species (Dickerson et al., 1994).

The National Academy of Sciences defines a biomarker or biological marker as a xenobiotically induced alteration in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample (Committee on Biological Markers, 1987). To this list may well be added xenobiotically induced alterations in behavior. Therefore, biomarkers can be broadly categorized as markers of exposure, effects, or susceptibility (ATSDR, 1994). The selection of appropriate biomarkers to be used for hazard evaluation is based on the mechanism of a chemically induced disease state. Moreover, growing awareness of the possibility of using wildlife as sentinels for human environmental disease has created a demand for biomarkers that are nonlethal and correlate with adverse effects in humans.

Dosing with an adequate concentration of a toxicant produces a continuum of responses beginning with exposure and perhaps resulting in the development of a disease. These events begin with external exposure, followed by the establishment of an internal dose leading to delivery of a contaminant to a critical site. This is followed either by reversible or irreversible adverse alterations to the critical site, resulting in the development of recognizable disease states. A clearer understanding of a chemically induced disease state in a species leads to an increase in the number of specific and useful biomarkers that may be extrapolated to other species. It is readily apparent that the earlier these effects can be measured at a critical site, the more sensitive the prediction of hazard or disease. However, in many cases the exact mechanism by which a toxicant induces injury is not well understood and nonspecific indicators of disease must be used.

Biomarkers of Exposure

The presence of a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured within a compartment of an organism can be classified as a biomarker of exposure (ATSDR, 1994). In general, biomarkers of exposure are used to predict the dose received by an individual, which can then be related to changes resulting in a disease state. In many cases, biomarkers of exposure are among the most convenient to determine because the contaminant or its metabolites can be quantified from nonlethally obtained samples of exhaled air, urine, feces, blood, or breast milk as well as tissues obtained through biopsy or necropsy. The former sources are the most desirable because they can be used for multiple determinations over time, thus making the biomarker more

useful by providing more information on the effects of the toxicant with time and by reducing variability.

Some very useful biomarkers of cancer involve detecting the ability of chemical carcinogens to form adducts with cellular macromolecules such as DNA or protein. Most chemical carcinogens are either strong electrophiles or are converted to an electrophilically active substance through metabolic activation (Miller and Miller, 1981). These carcinogens react with nucleophilic biomacromolecules to form adducts. If the biomacromolecule is sufficiently stable, adducts can then be detected by a variety of means and used to determine exposure profiles. Stable biomacromolecules can also provide measurement of the dose of a chemical carcinogen received by animals and humans. Adduct detection can be accomplished by total hydrolysis of the protein to alkylated amino acids (histidine, cysteine adducts), mild hydrolysis to release adducts (adducts that form esters to carboxyl groups or sulfonamides), immunodetection, or modified Edman degradation (adducts to N terminal valines on Hb). These techniques have been used to identify adducts formed by simple alkylating agents and their metabolites, aromatic amines, nitrosamines, and polynuclear aromatic hydrocarbons. One major advantage to this method of cancer risk determination is that blood samples are easily obtained and multiple samples can be obtained to determine patterns of exposure. In addition, the presence of adducts can often be detected by the creation of a point mutation. An example of this is the G-to-T transversion created following the formation of a N7 guanine adduct by benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide (BPDE) (Shibutani et al., 1993). Such point mutations can be detected by restriction fragment length polymorphisms.

Biomarkers of Effect

Biomarkers of effect are defined as measurable biochemical, physiologic, behavioral, or other alterations within an organism that, depending on their magnitude, can be recognized as an established or potential health impairment or disease (ATSDR, 1994). Ideally, a biomarker result must be able to stand alone. As such, it does not need chemical analysis or additional biological tests for confirmation. These tests are highly specific for individual chemicals and thus have a fairly limited application. Examples of such biomarkers include inhibition of brain cholinesterase by organophosphate or carbamate insecticides, induction of delta aminolevulinic acid synthetase and inhibition of aminolevulinic acid dehydratase by lead and certain other metals, and eggshell thinning by 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE), a metabolite of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) (Scott, 1977).

Less specific biomarkers are also well validated, but they have wider applications and tend to respond to broader classes of chemicals. Examples of these biomarkers are the induction of mixed-function oxidases, the formation of DNA adducts, other DNA alterations such as sister chromatid exchange and strand breakage, porphyrin profile alterations, induction of vitellogenin in oviparous vertebrates, and immunologic changes such as immunosuppression and hypersensitivity. These assays require either additional biomarker studies or chemical residue analysis in order to link causative agent to adverse effect. For example, the induction of cytochrome P4501Al (CYP1A1) enzymes in fish liver is generally recognized as a useful biomarker of the exposure of fish to anthropogenic contaminants, but these results are not compound-specific, as they may be induced by a variety of polynuclear and halogenated aromatic hydrocarbons as well as by hypoxia (HIF response element; Collier et al., 1995; Chan et al., 1999).

Finally, there is a long list of biomarkers that are under development or have been used with varying degrees of success but require further validation before they can be used in hazard evaluation. Thyroid function, retinol levels, plasma sex steroids, and stress proteins fall into this classification. Challenges exist in interpreting data from measurements of these endpoints because of normal circadian and seasonal variation, multiple known factors involved in the control of these endpoints, and marked interindividual variability.

Biomarkers of Susceptibility

Biomarkers of susceptibility are endpoints that are indicative of an altered physiologic or biochemical state that may predispose the individual to impacts of chemical, physical, or infectious agents. These biomarkers can be useful in predicting human disease states from wildlife sentinels. Low-level exposure to a cytochrome P4501A1 or 1A2 inducer, for example, may result in elevated enzyme activity in wildlife but no observable adverse effects. Such elevations in enzyme activity in humans have been linked to greater risk of a number of cancers due to increased bioactivation of procarcinogens. Similar observations have been made for decreases in conjugation enzymes and their high-energy substrates (Frame et al., 1998). In addition, a number of xenobiotic compounds inhibit the activities of the immune system and thus increase susceptibility to infectious agents, parasites, and cancer. Admittedly, the distinction between biomarkers of effect and susceptibility may be blurred. However, the distinction may be based upon whether the xenobiotic causes a physiologic or biochemical change that is directly indicative of a disease state or whether it reduces resistance to other biological, physical, or chemical agents.

Biomarker Interpretation

Caution must be used in interpreting biomarker results and extrapolating from one species to another. The same chemical may induce different proteins in one species when compared to another and the same enzyme may have different substrate specificities in species as closely related as the mouse and rat. For example, the common environmental contaminant *p,p'*-DDE induces cytochrome P4502B in the laboratory rat (*Rattus rattus*) but induces cytochrome P4501A1 in the deer mouse (*Peromyscus maniculatus*). Moreover, TCDD is a cytochrome P4501A1 inducer in the rat but induces both 1A1 and 2B in the deer mouse as determined by Western blotting, Northern blotting, and enzymatic activities (Nims et al., 1998, Dickerson et al., 1999). Similar differences exist between laboratory rats and birds, fish, and reptiles. Extrapolation of results requires a thorough knowledge of comparative physiology and biochemistry.

Alternatively, an important application of biomarkers is their ability to integrate multiple chemical exposures across an area with a variety of chemical contaminants, the scenario found at most chemical waste sites. CYP1A1 responses to sediments contaminated with dioxin, polychlorinated biphenyls (PCBs), or polynuclear aromatic hydrocarbons (PAHs) can provide insight to the status of the contaminants on site, their bioavailability, and the overall risk that they pose. Similarly, porphyrin profile alterations, metallothionein content, and immune function can provide insight to the combined effects of metals found on mine waste-contaminated

sites. It is thus essential in the use of biomarkers to understand both the strengths and the limitations of the techniques and to be cautious in extrapolating between species.

Beyond the current predominance of functionally based biomarkers, new trends in biomarker development appear distinctly molecular. A review of the most recent biomarker literature lists molecular biomarkers for a great many diseases and environmental contaminants ranging from secondhand smoke to suicide. The integration of biomarkers with epidemiology has resulted in a new discipline, molecular epidemiology, which has the potential for creating worldwide databases for environmental and genetic diseases (Albertini, 1999). The integration of biomarkers with molecular biology has revolutionized both medicine and biology by providing new tools by which to determine mechanisms of action (Costa, 1998). Moreover, these techniques can be applied to samples as small as one cell (Rao et al., 1998). Increasing emphasis is being placed upon nonlethal biomarkers such as enzyme-linked immunosorbent assay (ELISA) techniques for measuring fecal steroids in deer mice. A recent study defined four major needs in the development of biomarkers (Ward et al., 1996). New biomarkers are needed to monitor the continuum between exposure and overt disease. An increased knowledge is needed of the relationship between biomarker responses and disease pathology. In addition, better validation and increased sensitivity are required from existing biomarkers in order to better predict disease development. Last, as biomarkers become better tools for predicting environmental and genetic risk, a need to integrate science with policy emerges due to ethics of furnishing risk data to employers and insurance providers.

ENDOCRINE AND DEVELOPMENTAL DISRUPTORS

Endocrine disruption has recently emerged as a major issue, in terms of both science and public policy. A number of compounds, both natural and anthropogenic, cause alterations of the endocrine system (Colborn, 1996). Profound endocrine effects, both in individuals and at the population level, have been documented after exposure to high levels of certain compounds. All available evidence indicates that this issue will continue to evolve because of the controversial nature of the topic and the current insufficiency of data with which to make sound policy decisions (Kendall et al., 1998).

Mechanisms of Endocrine Toxicity and Sensitive Life Stages

It is evident that endocrine-disrupting compounds (EDCs) may interact with multiple targets. There is evidence for EDCs acting at every level of hormone synthesis, secretion, transport, site of action, and metabolism. Some examples of known mechanisms for EDCs include the following.

Receptor-Mediated Effects of EDCs A xenobiotic compound may exert effects at the receptor level through multiple mechanisms beyond the classic ligand-receptor interaction. These include differential effects at multiple receptor types or direct effects on intracellular signaling pathways, thereby directly influencing hormone action at the target tissue. Xenobiotic compounds may act on the endocrine system by affecting transcription and signal transduction and can act through receptor-mediated or nonreceptor-mediated mechanisms. For example, genistein has been shown to be a weak estrogen receptor agonist; however, it also modulates the activity of tyrosine kinases and DNA topoisomerases (Makela et al., 1994; Makela et al., 1995; Okajima et al., 1994; Olsen et al., 1994; Piontek et al., 1993; Whitten et al., 1995).

Effects of EDCs on Hormone Synthesis and Metabolism A compound may adversely alter levels of critical endogenous hormones by inducing or inhibiting biosynthetic or metabolic enzyme activities. Some phytoestrogens can interact with the 17β-dehydrogenase that regulates estradiol and estrone levels, suggesting that they can modulate overall estrogen levels in addition to acting as a ligand for the estrogen receptor. Perchlorate competitively inhibits thyroidal iodine uptake, thereby disrupting thyroid hormone synthesis (Lamm et al., 1999).

Effects on Hormone Secretion and Transport It has been known for many years that Cd^{2+} is a nonselective Ca^{2+} blocker that can disrupt Ca^{2+}-dependent exocytosis in hypothalamic neurosecretory neurons and pituitary endocrine cells, for example. Alternatively, EDCs can affect hormone-binding (sex hormone binding globulin, SHBG; corticosteroid binding globulin, CBG) proteins in blood, thereby disrupting hormone transport by increasing or decreasing the bound-to-free ratio of the hormone in plasma (reviewed in van der Kraak et al., 1998).

Timing of Exposure

There is substantial evidence that the sensitivity of an individual to gonadal steroids is dependent on the life stage of that individual. Specifically, the fetus appears to be the most sensitive life stage for lasting impacts of gonadal steroids or agonists/antagonists (Birnbaum, 1994; Blanchard and Hannigan, 1994; Ojasoo et al., 1992). For example, a compound may have little effect at environmentally relevant concentrations on a postpubescent animal but may prevent normal development if exposure occurs during fetal development or puberty. Research with polychlorinated biphenyls (PCBs) and dioxin has shown that gestational exposure is more critical than lactational exposure in eliciting developmental effects (Bjerke et al., 1994a; Bjerke and Peterson, 1994; Bjerke et al., 1994b). Sensitivity to EDCs is generally higher in fetal and perinatal individuals than in adults. However, in some cases, the presence of fetal serum-binding proteins may result in lower sensitivity to these compounds. For example, the ability of α-fetoprotein to bind 17β-estradiol protects the fetal male rat from maternal estrogen (Herve et al., 1990). Recent U.S. EPA workshops have identified the development of reproductive capability as the highest research priority in consideration of the features discussed above (Ankley et al., 1998).

Hormone Regulation and Feedback Control

There are several important control mechanisms that regulate estrogen biosynthesis during pregnancy. Estrogen levels are not feedback-regulated in a typical homeostatic mechanism; rather, there is a feed-forward mechanism resulting in steadily increasing serum levels of estradiol across most of pregnancy in rodents and humans (Casey et al., 1985). Thus, an exogenous dose of any estrogen agonist will be additive with the endogenous level because

feedback will not reduce endogenous production in a compensatory way. Additionally, in rodents and humans, the specific estradiol (E2) serum-binding proteins, α-fetoprotein (AFP), and testosterone-estradiol binding globulin (TEBG), also increase steadily during pregnancy, serving to protect the fetus from the high circulating estrogen level of pregnancy. Xenoestrogens that fail to bind effectively to these proteins have increased bioavailability (Sheehan and Young, 1979). Diethylstilbestrol and ethynylestradiol bind AFP with about 100-fold lower affinity than E2. Hence their bioavailability in newborn rats with high AFP levels is increased to about the same extent as E2, the bioavailability of which is decreased (Sheehan and Barnham, 1987). A fungal estrogen (i.e., Zearalenone) is about 0.066 percent as potent as E2 for adult uterotrophic responses, while equol, a plant estrogen, is about 0.25 percent as potent. In the neonatal rat, these numbers are 5 and 25 percent, respectively (Sheehan et al., 1984).

Species-Dependent Sex Determination

There are major differences in the control of sex determination among vertebrate classes. In mammals, sexual determination is based on the XY/XX system with the female as the homogametic sex. This system requires the synthesis of testosterone and dihydrotestosterone (through modification of testosterone by the action of 5α-reductase) in some target tissues and the presence of functional androgen receptors in the undifferentiated gonad, secondary sexual tissues, and brain (Norris, 1997). In rodents (but not necessarily in primates), the presence of estrogen receptors in the brain is essential for establishing male-type behavior. In order for this to occur, testosterone or a precursor must be aromatized to 17β-estradiol. Failure of any component results in the development of genetic males whose external phenotype or behavioral sex is not concordant with chromosomal sex (Norris, 1997). The sensitivity of this system is so exquisite that effects on reproductive development after in utero exposure may drive the risk assessment for EDCs (EC, 1996). In contrast, birds have a WZ/WW sex chromosomal system with the male as the homogametic sex. In birds, the ability to synthesize and recognize 17β-estradiol is necessary for female central nervous system (CNS) and gonadal sexual development to occur. A number of environmentally relevant chemicals can affect sexual differentiation and behavior in avian species. For example, masculinization of behavior in female birds may be observed following exposure to certain halogenated aromatic hydrocarbons (Fry, 1995; Nisbet et al., 1996; Rattner et al., 1984).

A nonheterogametic chromosome sex determination pathway exists in some reptilian species, predominantly the crocodilians, some turtles, and lizards. In these oviparous species, the temperature of incubation determines the sex of the embryo—a mechanism referred to as *environmental* or *temperature-dependent sex determination* (TSD). The window of sex determination for most animals is fairly narrow, comprising approximately 25 percent of the total incubation period (Norris, 1997). In some species, such as the American alligator (*Alligator mississippiensis*), the relationship between incubation temperature and sex is fairly linear, with lower incubation temperatures producing female offspring and higher incubation temperatures producing male offspring (reviewed in Matter et al., 1998). Moreover, incubation temperatures below 26°C and above 36°C result in embryonic death. For the red-eared slider (*Trachemys scripta*), the relationship is opposite. In other reptilian species, the relationship between sex and incubation temperature is more complex, with intermediate incubation temperatures producing predominantly male offspring and incubation temperatures on either extreme resulting in predominantly female offspring. The molecular mechanism of TSD is not well understood but may be the result of temperature-dependent control of aromatase (Rhen et al., 1999; Chardard and Dournon, 1999; Bergeron et al., 1999; Jeyasuria and Place, 1998). A number of compounds found in the environment can cause a reversal of sex determination in these species. Feminization of alligator and turtle embryos by DDE and hydroxylated PCBs has been reported (Guillette et al., 1999; Bergeron et al., 1994).

Endocrine disruption was initially observed in wildlife species and has received much attention in both the lay and scientific press. Although there are species differences in the response to EDCs, wildlife are sensitive to the effects of EDCs. Studies in wildlife are an important tool in determining the risk posed by EDCs in the environment. Table 29-1 lists a number of studies in various species, the causative agent (if known), and effects observed.

Further Issues on Endocrine and Developmental Toxicants

The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) was formed to develop strategies for evaluating the thousands of products and intermediates currently in use or in development that have the potential of human or environmental exposure. EDSTAC became necessary when the U.S. Congress mandated testing for endocrine-active substances in the Food Quality Protection Act (1996) and the Safe Drinking Water Reauthorization Act and Amendments (1996). These acts required that the U.S. EPA develop a screening program by August 1998, implement the program by August 1999, and report results back to Congress by August 2000. EDSTAC was chartered by the U.S. EPA administrator to provide advice and council to the U.S. EPA on these issues. This legislation increased the number of compounds likely to be tested from a few hundred to most chemicals in production or trials.

Currently, the most widely used tests are the Developmental Toxicology Test and Multigenerational Tests. These have been described previously in this volume. The limitations of the Developmental Toxicology Test are insufficient exposure during sexual differentiation and limited evaluation of reproductive and/or endocrine systems. Limitations of multigenerational tests include not enough diversity in the species tested, insufficient sensitivity of some endpoints, and failure to identify malformations elicited by known endocrine disrupting compounds (e.g., eggshell thinning).

EDSTAC recommended a two-tiered approach, with the first tier concerned with detecting—through the use of a battery of assays in mammalian and nonmammalian organisms—compounds that may be endocrinologically active, affecting the estrogen, androgen, and thyroid hormone systems. The second tier is designed to characterize the dose-response relationship of endocrine-disrupting compounds in wildlife and humans. Compounds are being selected (prioritized) for testing based upon their production volume, potential for exposure, result of high-throughput prescreening, structure, chemical class, and other relevant information. Once selected, the compounds will be evaluated by a series of in vitro and in vivo tests. These in vitro tests include estrogen receptor (ER) binding/transcriptional activation, androgen receptor (AR) binding/transcriptional activation, and steroid hormone synthesis using minced testis. Proposed in vivo tests include uterotrophic assay in adult ovariectomized rat, pubertal female rat assay including thy-

roid tests, (anti)androgen assay in castrate-T-treated male rat, frog metamorphosis assay for EDCs with thyroid hormone action, and a short-term fish gonadal recrudescence assay.

TERRESTRIAL AND AQUATIC ECOTOXICOLOGY

Many environmental studies include the analysis of contaminant exposure and effects on relatively small scales. However, contaminants can affect ecologic systems over large areas, including ecosystems and landscapes (Holl and Cairns, 1995). Ecosystems are composed of groups of all types of organisms that function together as well as interact with the physical environment, including energy flow and cycling of material between living and nonliving components (Odum, 1983). In turn, ecosystems collectively constitute landscapes with their own functional (nutrient and energy flow) and structural (patches, corridors) attributes. Movement of biotic and abiotic components within these large systems varies depending on several factors, including the species of animal and physical features of the system. Large vertebrates may roam over hundreds of square kilometers, integrating many habitat types within their home range. The area used by small animals may be small on an individual basis; however, dispersal individuals can maintain rather extensive connectivity among otherwise distinct local populations. Cycling and flow of materials maintain varying levels of connectivity within ecologic systems, such that disturbances to one component may be realized at another seemingly distinct component (Holl and Cairns, 1995). In general, ecologic systems are in a constant state of communication, which can potentially facilitate the large-scale effects of pollution.

Ecotoxicology includes all aspects of aquatic and terrestrial systems while attempting to elucidate the effects on biota following contaminant exposure. Exploring exposures to terrestrial systems and the effects of environmental contaminants within them is a recent endeavor relative to work that has been conducted historically in aquatic systems. Studies in aquatic and terrestrial toxicology rely heavily on interdisciplinary scientific exploration. Such research encompasses a variety of topics, including toxicity testing, sublethal responses of individual organisms, effects on populations and communities, and field research (Kendall and Lacher, 1994). A plethora of measurement endpoints exist that can be used to determine exposure and effects in different organisms or ecologic systems (Holl and Cairns, 1995; Melancon, 1995). These biological indicators of pollution may include individual-based measurements of some biochemical, physiologic, or morphologic endpoint (as previously discussed) or higher-order endpoint measurements including perturbations at population or higher levels. Thus, pollution may result in a cascade of events, beginning with effects on homeostasis in individuals and extending through populations, communities, ecosystems, and landscapes. This complexity and potential for large-scale effects extending through ecosystems results in a challenging research environment for environmental toxicologists.

Separation of aquatic and terrestrial environments in ecotoxicology is often impossible, as contaminants can be readily transported between these two systems. For example, contaminants in terrestrial environments may be transported to aquatic systems through surface runoff, resulting in exposure and effects in aquatic organisms located considerable distances from the source of contamination. Conversely, contaminants originating in aquatic environments may move into terrestrial environments following flood events or evaporation. One mechanism of contaminant movement of considerable interest in ecotoxicology is the transfer of contaminants through trophic levels, both within and between aquatic and terrestrial systems. Life-history strategies of many vertebrate and invertebrate organisms routinely integrate aquatic and terrestrial systems, resulting in exposure and effects scenarios that can be quite complex. Thus, although aquatic and terrestrial ecotoxicology are often considered separately, they are often intimately connected through abiotic and biotic mechanisms, examples of which can be found throughout the scientific literature.

Toxicity Tests

Acute and chronic toxicity tests are designed to determine the short- and long-term effects of chemical exposure on a variety of endpoints, including survival, reproduction, and physiologic and biochemical responses. Toxicity testing of terrestrial animal and plant species serves a number of purposes in terrestrial toxicology. Understanding the effects of a single compound provides a foundation for assessing the effects of contaminant mixtures. Because of the complex possibilities under typical field conditions, acute and chronic toxicity testing provides a critical foundation for evaluating the exposures and effects encountered in the field and for linking cause and effect to specific chemicals. For example, brain and plasma cholinesterase (ChE) inhibition has proven to be an excellent tool for monitoring exposure and in some cases for diagnosing the effect in animals exposed to organophosphate and carbamate pesticides (Mineau, 1991). Advances in toxicology have resulted in an expanding search for new sentinel plant and animal species for assessing contaminant exposure and effects. In turn, new sentinel prospects require testing to determine their sensitivity and the precision of their responses. Acute and chronic toxicity testing represents the initial steps toward validating new animal and plant species as useful sentinels of environmental contamination.

Results derived from acute and chronic tests can be used to determine the pathologic effects of contaminants, to provide data necessary to analyze the effects discovered in field tests, identify the potential effects to be aware of under field conditions, and provide dose-response data for comparison to exposure levels in the field. Although they measure effects at the individual level, acute and chronic toxicity tests were designed for the purpose of protecting natural ecosystems from perturbation due to anthropogenic contamination. There are concerns raised by some researchers that laboratory toxicity tests are not realistic predictors of effects in complex field ecosystems. On the other hand, others have argued that short-term toxicity data provide conservative indices by which to judge potential effects of chemicals and effluents on natural populations and ecosystems (Cairns and Mount, 1990). It has even been found that toxicity tests can sometimes be used as indicators of potential effects on community structure (Norberg-King and Mount, 1986; Hartwell, 1997).

Sublethal Effects

Mortality represents a nonreversible endpoint of interest in ecotoxicology. However, documenting die-offs can be challenging, as success is affected by search efficiency and rapid disappearance of carcasses (Rosene and Lay, 1963). Also, many contaminants exist in smaller, nonlethal amounts or in relatively unavailable forms, such that acute mortality is unlikely. Thus, understanding and monitoring the sublethal effects of contaminant exposure in

aquatic and terrestrial systems is of great interest. The existence of sublethal effects in exposed organisms has been used as an advantage in monitoring strategies. Biochemical and physiologic measurement endpoints have been developed or adapted from other sources and, in turn, used with various plant and animal sentinels to assess exposure and effect in many different species (Lower and Kendall, 1990; Kendall et al., 1990; Huggett et al., 1992; Adams et al., 1992; Theodorakis et al., 1992). Inhibition of ChEs has proven an excellent marker that is both sensitive and diagnostic for organophosphate and carbamate insecticide exposure (Mineau, 1991). Induction of enzyme systems, such as the mixed-function oxygenases, are also useful as sublethal biomarkers of exposure to many types of environmental pollutants (Elangbarn et al., 1989; Rattner et al., 1989). Other strategies for monitoring sublethal effects include monitoring immune function (McMurry et al., 1995), genotoxicity (McBee et al., 1987), and reproductive endpoints (Kendall et al., 1990). Even though these effects may not result in immediate mortality, they can affect fecundity and reproductive success of aquatic and terrestrial organisms and ultimately have effects on population structure and function. Chemicals may also affect the growth rate of organisms. Because growth rate and body size are related to reproductive maturity in juvenile organisms and its attainment as well as relative fecundity in adults, chemical stressors that inhibit growth rates can also affect the reproductive potential of the population.

Sublethal effects of contaminant exposure reach beyond the intrinsic physiologic and biochemical responses to many behavioral traits of the individual. Decreased predator avoidance capability may expose individuals to increased susceptibility to predation (Bildstein and Forsyth, 1979; Preston et al., 1999). Foraging behavior may be altered by chemicals, such that foraging efficiency or success in prey capture is diminished (Peterle and Bentley, 1989; Smith and Weis 1997). Migration and homing also may be affected, decreasing the general fitness of the individual (Snyder, 1974; Willette, 1996; Vyas et al., 1995). Altered breeding behavior may decrease fecundity through impaired nest-building and courtship behavior, territorial defense, and parental care of the young (McEwen and Brown, 1966; Jones and Reynolds, 1997). In addition, changes in fish behavior patterns or avoidance of contaminated water have been used as indicators of aquatic pollution (Gruber et al., 1994; DeLonay et al., 1996). These may occur at earlier times or at lower doses than overt mortality, providing an early-warning indicator of toxic effects (Gerhardt, 1998).

Determination of sublethal effects is an important component of risk assessments for two reasons. First, these responses may provide information not available from measurements of contaminant tissue concentrations. This is because (1) it may not be possible to measure tissue concentrations of some chemicals because they are rapidly metabolized and (2) the toxic effects of many chemicals, especially when present in complex mixtures (as is usually the case in the environment) may not be predicted from tissue concentrations alone (Lower and Kendall, 1990). Second, alterations of biochemical and molecular physiology have been associated with reductions of fecundity, growth, and bioenergetic status of affected organisms (Adams et al., 1989, Theodorakis et al., 1996, Steinert et al., 1998). Hence, perturbations of subcellular function may affect fitness and health of fish and wildlife, and may ultimately be translated as effects on populations and communities.

Although they are quite similar, sublethal effects in aquatic and terrestrial organisms do differ in some important aspects. For example, there are a number of suborganismal (cellular, molecular, histologic) effects that can be detected in aquatic and terrestrial organisms, and many of these are commonly studied in both types of organisms—e.g., liver mixed function oxidase induction (Goksøyr and Förlin, 1992). However, aquatic and terrestrial organisms may differ in the relative magnitude of these responses. DNA-repair enzyme activity may be lower in fish than in mammals (Wirgin and Walden, 1998). Additionally, some toxicant-responsive genes in terrestrial vertebrates may not have homologs in aquatic organisms, possibly leading to species-specific differences of toxic effect or induction of these genes (Hahn et al., 1992). There are also differences between aquatic and terrestrial biomarkers in relation to the attention given to various endpoints. For example, studies that examine acetylcholinesterase inhibition mainly focus on terrestrial organisms, whereas studies examining DNA damage (Shugart and Theodorakis, 1994) and metallothionein induction (Roesijadi, 1992) are more heavily represented in aquatic studies. Another class of protein that can be induced by contaminant exposure are the stress or "heat-shock" proteins (Sanders, 1993), which participate in the renaturation of damaged proteins. Although they are highly conserved in all organisms from bacteria to mammals and are a major focus of study in the biomedical field, in the field of ecotoxicology, studies on the induction of stress proteins focus almost exclusively on aquatic organisms. Aquatic toxicology studies also differ from those in terrestrial toxicology because aquatic organisms respire through gills. Gills may be constantly exposed to water-borne contaminants and are highly permeable to dissolved substances. As a result, gills may accumulate certain contaminants (Robinson and Avenant-Oldewage, 1997) or their structure and function may be impaired (Karan et al., 1998; Li et al., 1998). Conversely, terrestrial organisms will realize most of their exposure through ingestion of contaminated media.

Population and Community Effects

One of the major objectives of ecotoxicology is the detection and prevention of pollutant effects on population structure and function. These effects may be determined by collection of empiric data or simulated with the use of population models (Albers et al., 2000). In the former case, natural populations are sampled in order to determine the effects of environmental contamination on density, abundance, or biomass of indigenous organisms (Rask, 1992; Welsh and Ollivier, 1998). These values from contaminated populations are then compared with those from reference populations (with no history of contamination) in order to determine pollution effects. Such effects may also be manifest as changes in age structure or sex ratios, which may affect the reproductive potential of the population (DeAngelis et al., 1990). The age structure of populations (relative number of individuals of each age class) may give an indication of pollutant effects, such as reproductive failure or perturbations in recruitment of juveniles into the population (Vuori and Parkko, 1994; Hesthagen et al., 1996). The pattern of population response to pollution may also provide information as to the mechanism of population effect, such as changes in adult mortality, juvenile recruitment, food availability, etc. (Gibbons and Munkittrick, 1994).

Alternatively, effects of pollutants on populations can be predicted or simulated using mathematical models. These models use empiric data such as abundance, age distribution, and age-specific mortality and fecundity in order to predict effects of pollutant exposure on abundance of individuals and rate of population change

(growth or decline). The empiric data are gathered from organisms grown in laboratory cultures or from natural populations, and population parameters are calculated using linear or matrix algebra (DeAngelis et al., 1990). Models also exist that use toxicity test data derived from laboratory exposures in combination with population parameters in order to predict effects of pollutants on populations (Barnthouse et al., 1990). Other models use physiologic and behavioral parameters of individuals in order to predict such effects (DeAngelis et al., 1990).

Any effects on populations may ultimately be manifest as effects on communities because, by definition, communities are collections of interacting populations. Environmental contaminants can affect the structure of communities as well as the interactions of species within them. For example, it is well known that exposure to chemicals may cause a reduction of community diversity (relative number of species) and changes in community composition (e.g., LaPoint et al., 1984; Hartwell et al., 1997; Beltman et al., 1999). In addition, the trophic structure of fish and invertebrate communities may also be affected by exposure to anthropogenic chemicals (Camargo, 1992; Paller et al., 1996).

The trophic structure of communities is related to the relative abundance of species that feed on various food items (piscivores, omnivores, detritivores, insectivores, etc.) or have various foraging methods (shredders, scrapers, etc.). These changes in species/trophic composition may come about by direct or indirect mechanisms. The direct effects involve loss of some species due to an increase in pollution-induced mortality or reduced reproductive output. In this case the communities will be dominated by species that are less affected by pollutant exposure. This is the basis of a phenomenon termed *pollution-induced community tolerance,* or PICT (Blanck and Wangberg, 1988), in which algal communities become more pollution-tolerant over time due to the replacement of pollution-sensitive species with more tolerant ones. Some evidence of this phenomenon has also been observed in terrestrial systems where shifts in the composition of rodent communities appears to indicate contaminant-induced reductions of select species in the community in favor of resistant or resilient species (Allen and Otis, 1998).

Alternatively, community structure may change through indirect mechanisms. For example, a species may be absent from a community because the organisms upon which it feeds are exterminated by pollutant exposure. Indirect effects may also be affected by changes in dynamic interactions between species—for example, predator/prey interactions. Analogously, if competing species differ in relative sensitivity to a pollutant, environmental contamination may give one species a competitive edge over the other, resulting in local extinction of the less tolerant species. Finally, it has been suggested that such changes in community structure come about because some species are more genetically adaptable than others and so are better able to adapt to novel stressors such as pollution (Luoma, 1977). Thus, the more sensitive species would not be able to adapt to this stressor and become locally extinct. These types of perturbations in community structure and dynamics may ultimately compromise the stability, sustainability, and productivity of affected ecosystems.

Chemical Interactions and Natural Stressors

As more information becomes available on chemical effects in aquatic and terrestrial organisms, there is increasing interest in understanding the interactive effects of exposure to multiple contaminants as well as the interactions between contaminants and inherent stressors (e.g., nutritional stress, disease, predation, climate, water quality). This area of ecotoxicology is one of the least understood because of the a priori need to understand the more direct exposure and effects scenarios. Nevertheless, it represents an expanding part of ecotoxicology and is generating interest in the research community.

Perhaps the greatest inherent stressors faced by many species of wildlife are nutritional restriction and seasonal shifts in climatic extremes. Daily food restriction of as little as 10 percent below normal intake has been shown to enhance the overall decline in courtship behavior, egg laying and hatching, and number of young fledged by ringed turtle doves (*Streptopelia risoria*) exposed to DDE (Keith and Mitchell, 1993). Antagonistic relationships also exist. Methionine supplementation effectively negated the detrimental effects of selenium toxicity on mortality in mallard ducklings (Hoffman et al., 1992). Similarly, relative magnitude of biomarker responses and tissue distribution of contaminants in fish may be influenced by nutritional status and food deprivation (Joergensen et al., 1999). Effects have also been found for the interaction between temperature and chemical exposure. Cold stress has been shown to augment the effects of pesticide exposure, resulting in increased mortality of several wildlife species (Fleming et al., 1985; Rattner and Franson, 1984; Montz and Kirkpatrick, 1985). However, more subtle interactive effects on energy acquisition and allocation were less conclusive in deer mice exposed to aldicarb—2-methyl-2-(methylthio)propanal *O*-[(methylamino)carbonyl] oxime—and cold stress (French and Porter, 1994). Unlike many wildlife species, fish and aquatic invertebrates are poikilotherms, so their metabolic rate is more dependent on ambient temperature than that of birds or mammals. As a result, toxicity, accumulation, and metabolism of aquatic contaminants may be influenced by water temperature (Odin et al., 1994; Sleiderink et al., 1995; van Wezel and Jonker, 1998). Other environmental variables, such as salinity and pH, may also affect uptake and toxicity of aqueous chemicals (Norrgren et al., 1991; Hall and Anderson, 1995). Conversely, exposure to pollutants may affect an organism's ability to tolerate natural environmental variables such as water oxygen concentrations (Bennett et al., 1995). Other areas of interest include interactions between chemical exposure and social stress (Brown et al., 1986) and interactions between different chemicals (Stanley et al., 1994).

Trophic-Level Transfer of Contaminants

Although contaminant exposure may occur through inhalation, dermal contact, or ingestion from preening or grooming behavior, significant exposure also occurs through food-chain transport. Depending on specific chemical properties, contaminants may accumulate in either soft or hard tissues of prey species. Species not normally in direct contact with contaminated media may become exposed through ingestion of contaminated prey, promoting accumulation or magnification of contaminants into higher trophic levels. Earthworms in soils contaminated with organochlorines and heavy metals can accumulate quantities of contaminants known to be deleterious to sensitive species (Beyer and Gish, 1980; Beyer and Cromartie, 1987). The use of pesticides to control plant pests often coincides with the reproductive periods of many wildlife species, enhancing exposure potential in juveniles that often rely on invertebrates as a primary food source (Korschgen, 1970).

The foraging habits of individual species dictate the potential for contaminant exposure through food-chain transport. In a field study in Canada, Daury and coworkers (1993) found a higher percentage of ring-necked ducks (*Aythya collaris*) with elevated blood lead concentrations compared to American black ducks (*Anas rubripes*). The difference was attributed primarily to foraging habits, as ring-neck ducks are divers and may consume up to 30 percent invertebrates in their diet, compared with American black ducks, which forage on the surface of the water. Even when contaminated prey is ingested, exposure may be minimal in certain species. Adult American kestrels (*Falco sparverious*) fed pine voles (*Microtus pinetorum*) with mean body burdens of 48 μg/g DDE, 1.2 μ/g dieldrin, and 38 μg/g lead accumulated approximately 1 μg/g lead in bone and liver tissue but 232 μg/g DDE and 5.9 μg/g dieldrin in carcasses after 60 days. Mean lead concentration in regurgitated pellets from kestrels was 130 μg/g, demonstrating their lack of lead accumulation from contaminated prey (Stendell et al., 1989). Secondary poisoning from food-chain transfer has also been implicated in the mortality of endangered species. Lead poisoning was apparently responsible for the deaths of several California condors (*Gymnogyps californianus*) found in California. The probable source of the lead was considered to be bullet fragments consumed by condors feeding on hunter-killed deer (Wiemeyer et al., 1988).

The potential exposure of predatory species may be enhanced by the altered behavior of contaminant-exposed prey. Affected prey may be easier to catch, leading predators to concentrate their foraging efforts on contaminated sites and thus increasing their direct exposure and the transfer of contaminants through trophic levels (Bracher and Bider, 1982; Mendelssohn, 1977). As contaminants move through food chains, they may be translocated from their source. Migrating individuals may transport contaminants considerable distances, resulting in potential exposure and effects in organisms that otherwise would not be in contact with contaminated sites (Braestrup et al., 1974).

Genotoxicity

Ecogenotoxicology is a relatively young field that has benefited tremendously from the growth of molecular biology and molecular genetics. It is concerned with the effects of pollutants or chemicals (mutagens, clastogens, aneuogens, and teratogens) on the genetic material of organisms. Such genetic material is usually defined as DNA, RNA, and chromosomes but may also include modifications of proteins. Such effects may be manifest as DNA strand breaks, base modifications, chromosomal rearrangements or fragmentation, and aneuploidy (Shugart and Theodorakis, 1994). While it is possible to damage the genetic material of an organism without any subsequent effect on that individual, it is also possible that mutations in the DNA can result in somatic effects such as cancers. If these effects occur in germinal tissues, this can also result in heritable effects and an increase in the genetic load (i.e., relative frequency of deleterious mutations in the population). Other types of multigenerational effects may not occur by direct interaction of contaminants with the DNA molecule but by selection pressure from chemical contaminants. Because this can change the evolutionary nature of a species, Bickham and Smolen (1994) coined the phrase *evolutionary toxicology* to describe this phenomenon. They proposed that selection resulting from the stress of somatic effects of contaminants could lead to population genetic changes that are not predictable from a knowledge of the mechanisms of toxicology of the contaminants. Also, individuals that have the pollutant-resistant genotypes may be more susceptible to natural stressors (Weis et al., 1982). Furthermore, because changes in the genetic makeup of the population involve alterations in survival and recruitment, such changes may be indicators of adverse chronic effects on population structure and dynamics. Selection for pollutant-resistant genotypes, as well as genetic bottlenecks—a result of reductions in population size or recruitment—may reduce genetic variability in affected populations (Guttman, 1994; Theodorakis and Shugart, 1998). These effects may be indicators of community-level effects, because it has been found that patterns of genetic diversity and community-level pollution effects are correlated in contaminated streams (Krane et al., 1999). These were termed *emergent* effects.

Besides selection and genetic bottlenecks, an elevated mutation rate may also alter population genetic structure. The search for methods to detect mutations easily among millions of base pairs is one of the primary needs of genotoxicology. The mitochondrial DNA has the least effective repair mechanism and should be among the fragments of DNA that permit detection of an elevated mutation rate. However, it is often difficult to detect an increase in the mutation rate because baseline mutation rates are so low that even highly contaminated environments may fail to induce significant changes (reviewed in Cotton, 1997). For example, studies of Chernobyl mice experiencing doses in excess of 15 rads per day (Chesser et al., 2000) failed to detect statistically significant elevation of mutation rates (Baker et al., 1999). In addition, minisatellite and microsatellite mutation frequencies are among the highest documented for the nuclear genome, and this phenomenon appears to have potential in genotoxicology. Makova et al., (1998), however, failed to find an elevated mutation rate in mice at Chernobyl. Dubrova et al. (1996) reported an elevated mutation rate in minisatellite loci in children born to survivors of the Chernobyl disaster.

It is appealing to use native species living in a highly polluted environment to determine multigenerational effects on the genome (McBee and Bickham, 1990). The basic assumption is that living in a polluted environment will result in reduced fitness and deterioration of health of the sentinel species. With an adequate array of biomarkers such as alterations in the DNA (Shugart et al., 1994), mini- and microsatellites (Dubrova et al., 1996; Bickham et al., 1998), micronuclei frequency (Heddle et al., 1991, MacGregor et al., 1995; Rodgers and Baker, 2000), flow-cytometry values (Bickham et al., 1992), enzymatic assays (Jensen et al., 1997; Langlois et al., 1993), and population genetic characteristics (Matson et al., 2000), it should be possible to estimate risk and genotoxicologic damage.

However, the issue is not simple because life is resilient and often highly polluted environments are modified and devoid of other human activities. Some areas with extremely high levels of radioactivity, like Chernobyl, may support population densities and levels of biodiversity reminiscent of conservation parks, suggesting that human activities can be more detrimental to natural ecosystems than the world's worst nuclear power plant disaster. The problem of using native species as sentinel species may be further complicated by adaptation of the local populations to the polluting chemicals (Theodorakis et al., 1998). Undoubtedly, studies that resolve reduced fitness and health issues of wildlife will require excellent experimental design using control populations and multigenerational data.

Terrestrial Ecotoxicology

Terrestrial toxicology is the science of the exposure to and effects of toxic compounds in terrestrial ecosystems. Investigations in terrestrial toxicology are often complex endeavors because of a number of intrinsic and extrinsic factors associated with terrestrial systems. All organisms function at several levels, from the individual level to the level of the ecosystem, interacting with others within the constraints of social ranking, food webs, and niches. Many terrestrial species are very mobile, covering significant areas while defending territories, foraging, migrating, and dispersing. Terrestrial toxicology includes all aspects of the terrestrial system while attempting to elucidate the effects on the biota following contaminant exposure. Exploring exposures to and the effects of environmental contaminants in terrestrial systems is a recent endeavor relative to work that has been conducted historically in aquatic systems. Like aquatic toxicology, however, terrestrial toxicology relies heavily on interdisciplinary scientific exploration.

The early 1900s witnessed the relization that chemicals used in the environment could affect nontarget organisms. Studies were conducted on the exposures to and effects of arsenicals, pyrethrums, mercurials, and others on terrestrial organisms (Reviewed in Peterle, 1991). In later years, synthetic pesticides became increasingly important in controlling pest species in agricultural crops, although little was known about their effects on nontarget organisms. As pesticide development and use continued, however, reports of wildlife mortalities and declining avian populations spawned concern among biologists internationally. Studies were conducted that documented residues of DDT and DDT metabolites, other chlorinated hydrocarbon insecticides, and industrial chemicals, including PCBs, in the tissues of wildlife species. Although reduced nesting success was apparent in some avian species (e.g., osprey, bald eagles, Bermuda petrels, herring gulls, and brown pelicans) (Ames, 1966; Peterle, 1991; Wurster and Wingate, 1968; Keith, 1966; Schreiber and DeLong, 1969), the underlying mechanism was not completely understood until later.

The study of the toxic effects of chemicals on terrestrial organisms witnessed its most dramatic growth in the 1980s (Kendall and Akerman, 1992). Requirements for detailed and accurate information on the effects of pesticides on terrestrial wildlife species played a large part in the development of terrestrial toxicology methodologies. Persistent pesticides such as DDT and mirex [1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-methano-1*H*-cyclobuta(cd)pentalene] were shown to accumulate in wildlife species. Development of new insecticides, such as organophosphates, lessened the problem of persistence, although toxicity was still a concern. An obvious need existed by which scientifically sound investigations could be conducted to explore the direct and indirect effects of chemicals on terrestrial wildlife populations.

Chemical effects on avian populations were the primary focus for many years. This problem became more apparent as the link was established between DDT contamination and declining bird populations. The classic case of eggshell thinning in raptor eggs was established by Ratcliffe (1967) in studies on declining sparrow hawk (*Accipiter nisus*) and peregrine falcon (*Falco peregrinus*) populations in the United Kingdom. Other studies soon followed and it became apparent that many avian species suffered reduced productivity resulting from eggshell thinning and decreased hatching success. Studies continue to be conducted on the exposure and effects of these persistent pesticides in wildlife species (Bergman et al., 1994; Custer and Custer, 1995; Auman et al., 1997; Allen and Otis, 1998; Elliott and Norstrom, 1998, Creekmore et al., 1999).

Acute and Chronic Toxicity Testing Terrestrial organisms are typically exposed to contaminants through ingestion of some contaminated media, although inhalation and dermal absorption of contaminants do occur. Thus, toxicity tests for terrestrial species are usually designed to test the effects of a chemical dose, administered by oral gavage or injection. Exposure can also be accomplished through consumption of contaminated food or water, resulting in dosages calculated from consumption rates or simply exposure over time to a given concentration of contaminant in the diet. Methods for measuring endpoints in toxicity tests include the LD_{50} and LC_{50}, the ED_{50} and EC_{50}, and reproductive tests (fertility, egg hatchability, neonate survival). These endpoints can be used to assess toxicity in a variety of terrestrial animals, including earthworms (*Eisenia foetida*), honeybees (*Apis mellifera*), northern bobwhite, mallards, mink, and European ferrets (*Mustela purofius furo*) (Menzer et al., 1994). Likewise, specialized tests for determining toxicity in plants are used to assess lethal and nonlethal response to contaminants. Standardized tests for toxicity in plants include germination assays for lettuce seeds (*Latuca sativa*), root elongation in seedlings, and analysis of whole plants such as soybean (*Glycine max*) and barley (*Hordeum vulgare*) (Wang, 1985; Greene et al., 1989; Pfleeger et al., 1991; Ratsch, 1983). Other plant and animal species, including domestic and wild types, can be used in standardized testing systems as dictated by specific site requirements (Lower and Kendall, 1990).

Standardized laboratory toxicity tests performed under U.S. EPA guidelines include acute oral LD_{50}s and dietary LC_{50}s on northern bobwhite quail and mallard ducks. Also, mammalian toxicity tests include acute oral LD_{50}s on rats using estimated environmental concentrations of the chemical in question. Avian and mammalian reproductive toxicity testing may be required under certain circumstances, depending on such factors as food tolerance, indications of repeated or continued exposure, the persistence of chemicals in the environment, and chemical storage or accumulation in plant or animal tissues (U.S. EPA, 1982).

Field Testing Field studies are designed to address exposure to contaminants and resulting effects to organisms outside the highly controlled environment of the laboratory. Field studies may be designed specifically to address concerns suggested by laboratory studies or to test modeled or predicted exposure and effects based on site contaminant levels. As the effects of environmental contaminants on wild populations of animals have become more apparent, the need for more useful field testing methodologies has led to improved assessment strategies. Whether the U.S. EPA requires field testing depends both on laboratory testing results, professional judgment, or the degree of consensus on anticipated exposure and effects. Chemical properties of the compound, intended use patterns (e.g., pesticides), difference between the estimated environmental concentration (EEC) and the lowest observed effect level (LOEL), and dose-response relationships are considered in combination when exploring the need for conducting field studies.

Field studies are conducted in complex ecologic systems where plants and animals are affected by numerous natural stres-

sors (e.g., nutrient restriction, disease, predation) that might possibly confound the measurement of contaminant exposure and effects. In addition, life history characteristics vary dramatically among species. Issues of habitat use, home range size, foraging characteristics, and other factors must be considered in designing a field study. Field study design must be robust to noncontaminant influences, and some important considerations include censusing techniques, sampling units, site replication, scale ecologic similarity among sites, and choice of study organisms. Results from several studies indicate the potential complexity involved with censusing animals exposed to contaminants, as alterations in behavior and observation difficulties may bias results (Grue and Shipley, 1981; Fryday et al., 1996; Hawkes et al., 1996; Madrigal et al., 1996).

Traditional methods used by biologists and wildlife ecologists have been used successfully in terrestrial ecotoxicology field studies, and resources are available that describe the various techniques for trapping, remote sensing, and sampling terrestrial biota (Bookhout, 1994; Menzer et al., 1994). Ligature techniques used for birds have improved the process of collecting food from nestlings raised on contaminated sites, allowing researchers to better determine the composition of the diet and to ascertain the contaminant loads in foodstuffs (Mellott and Woods, 1993). The published results of field studies have provided information on the impacts of contaminants on wildlife abundance and survival (Rowley et al., 1983), acute mortality (Babcock and Flickinger, 1977; Kendall et al., 1992), food-chain relationships (Korschgen, 1970), reproduction (Clark and Lamont, 1976; Hooper et al., 1990), and behavior (Grue et al., 1982). Basic laboratory techniques are often integrated with field methods to determine the ecologic significance and mechanisms of exposure and effects on populations (Hooper et al., 1990).

Techniques for the assessment of wildlife exposure and its effects must incorporate sufficient flexibility to allow their use on sites with a wide variety of physical and chemical characteristics (Fite et al., 1988; Warren-Hicks et al., 1989). To accomplish this goal, three approaches to wildlife assessments are generally used to provide the required breadth. These are the use of (1) endemic species occurring naturally on contaminated sites, (2) enhanced species attracted to the site by creating more favorable breeding habitat, and (3) enclosed species derived from clean laboratory-bred populations (Hooper and La Point, 1994).

Field studies are often designed to study populations of organisms living on contaminated sites, which are then compared with other populations living on noncontaminated reference sites. The primary benefit in these studies is the use of endemic species that receive lifelong exposures to site contaminants of concern. Detracting from the utility of these studies is the lack of control over such factors as exposure history or genetic background of individuals. Although some control is available over other factors such as the test species and habitat type, study design is still subject to the local conditions dictated by the contaminated site. Further, the small sizes of some sites can preclude effective use of some native wildlife species that roam over large areas.

Enhanced species studies generally include assessing the reproductive effects of contaminant exposure on species that inhabit nest boxes, such as the European starling (*Sturnus vulgaris*), which provides a model for assessing other cavity-nesting passerines with similar life history traits (Kendall et al., 1989). Cavity-nesting birds readily occupy artificial nest cavities and will often colonize study sites when provided with nest boxes. Increased numbers of adults and nestlings are thus available, from which information on reproductive success, behavioral response, exposure routes, and physiologic and biochemical perturbations can be obtained during the breeding season. Numerous studies have taken advantage of these traits in other avian species, including eastern bluebirds (*Sialia sialis*), American kestrels, and—more recently—tree swallows (*Tachycineta bicolor*). Tree swallows have been used extensively to assess exposure and effects from a number of contaminants (Shaw, 1984; DeWeese et al., 1985; Custer et al., 1998; Bishop et al., 1999, McCarty and Secord, 1999).

Use of enclosures has greatly enhanced control over many of the environmental factors that can complicate field studies. Enclosure studies incorporate a variety of outdoor, open-air facilities to enclose test organisms during toxicologic testing. The purpose of using enclosures is to simulate natural field conditions while maintaining a level of control over experimental conditions (e.g., exposure period, nutritional condition, test organism, sex ratios, age, genetic similarity, habitat type). In essence, enclosure-based experiments can be used to bridge the gap between laboratory and field investigations. Study organisms are more readily accessible when housed in enclosures, making it easier to take multiple samples from individuals, administer treatments to them, and monitor their behavior and reproduction. The flexibility afforded under these conditions makes it possible to explore a number of questions regarding the potential interactions between the contaminant and natural stressors in the environment. Enclosure studies may be required by the U.S. EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) guidelines for pesticide registration if they can potentially yield useful information about pesticide impacts on wildlife.

Enclosure studies have been used successfully with aquatic and terrestrial species to explore the effects of pesticides and chemical contaminants on abundance, reproduction, immune function, and biochemical response (Barrett, 1968; Pomeroy and Barrett, 1975; Barrett, 1988; Dickerson et al., 1994; Gebauer and Weseloh, 1993; Weseloh et al., 1994; Hooper and La Point, 1994; Edge et al., 1996; Caslin and Wolff, 1999). Basic approaches to using enclosures to study the impacts of chemicals on terrestrial organisms vary widely. There is considerable variation in enclosure size; they range from less than 1 m^2 to more than a hectare. Small stainless steel enclosures, approximately 0.5 by 1.5 in, have been used to house laboratory-raised deer mice (*Peromyscus maniculatus*) for the assessment of contaminant uptake and biomarker response in mice on sites contaminated with polynuclear aromatic hydrocarbons (Dickerson et al., 1994). Larger enclosures can be used to monitor population-level responses and community interactions. Barrett (1988) and Pomeroy and Barrett (1975) used large enclosures to assess population and community responses of several rodent species to controlled applications of Sevin (1-naphthyl-*N*-methylcarbamate) insecticide. Studies incorporating pinioned ducks on contaminated waste ponds provide an equivalent method for avian species (Gebauer and Weseloh, 1993; Weseloh, 1994).

Although large enclosures offer the advantage of addressing population- and community-level issues of toxicant effects, they can be restrictive in cost and space. Smaller enclosures are affordable and can be beneficial for site-specific evaluations. They can be easily moved among locations, making them an excellent strategy for short-term testing and determining the efficacy of site remediation. However, the design of enclosures depends on the goals of the study.

Aquatic Ecotoxicology

Aquatic toxicology is the study of effects of anthropogenic chemicals on organisms in the aquatic environment. The aquatic ecosystem is of particular concern because this is where most contaminants released into the environment are eventually deposited, either from direct discharge into bodies of water or from terrestrial runoff and atmospheric deposition (Pritchard, 1993). Furthermore, there are certain features of the aquatic environment that make it unique. First, certain chemicals are not volatile in air but are soluble in water (e.g., metals), so aquatic organisms may be exposed to chemicals via routes that are not present in their terrestrial counterparts. Also, many contaminants are readily degraded in an aerobic environment, but the aquatic environment frequently contains little or no oxygen. Therefore, some contaminants can persist in aquatic ecosystems far longer than in terrestrial systems (Ashok and Saxena, 1995). Finally, aquatic organisms are frequently restricted in their habitat and home range, so they often cannot avoid contaminated areas. These attributes of aquatic systems present unique circumstances and problems that are not applicable to terrestrial systems.

The physiology and anatomy of aquatic organisms can also present problems that are different from those faced in the study of terrestrial organisms. For example, aquatic organisms have highly permeable skin and gills and so are particularly susceptible to the effects of ambient contamination (Pritchard, 1993). Furthermore, aquatic communities are dominated by ectothermic organisms (e.g., invertebrates, fish, amphibians, and, to a lesser extent, aquatic reptiles), whose metabolic rate is determined by ambient water temperature. Thus, the accumulation of contaminants and their toxic effects are influenced by water temperature, which can vary both spatially and temporally. Finally, fish and amphibians are unique among the vertebrates in that they have a highly permeable anamniotic egg (an egg without a shell or amniotic membrane), and the embroyo develops while the egg is completely immersed in water. They are also unique in that they are the only vertebrates that have an aquatic larval life stage that undergoes metamorphosis. For these reasons, the embryo/larval stages are often very sensitive to chemical insult and may be vulnerable to effects experienced by terrestrial vertebrates.

Acute and Chronic Toxicity Testing In aquatic toxicity tests, fish, invertebrates, or algae are exposed to aqueous chemicals in the laboratory. Designs for these tests include static (in which the test water is not renewed for the duration of the test), static renewal (test water is renewed periodically), and flow-through systems (test water is renewed continually). The organisms may be exposed for short (acute toxicity tests) or long periods (chronic toxicity tests). Static designs are usually restricted to acute toxicity tests, and chronic tests frequently have a flow-through design. Acute and chronic tests not only differ in duration but also in the endpoints that are measured. In acute tests, survival is often the only endpoint (ASTM, 1992a). However, in chronic tests, effects on growth and reproduction are also determined. To accomplish this, the duration of the chronic test is designed to span the entire life cycle of the organism (i.e., from zygote to age of first reproduction; U.S. EPA, 1989). However, chronic tests are often difficult to perform because of their long duration (9 to 30 months for fish tests) and are very expensive, which makes their routine use prohibitive. Hence, three alternatives to full life-cycle tests have been developed: partial life-cycle tests, early-life-stage tests, and short-term chronic tests. Partial life-cycle tests are used for fish that require >12 months to reach reproductive maturity. They begin with immature fish prior to gonadal maturation and end after the first reproduction in order to determine the effects of aquatic contaminants on reproductive potential of the fish. Early-life-stage tests determine toxicity in fish exposed from the embryonic through juvenile stages and are typically 1 to 2 months in duration (ASTM, 1992b). The rationale is that the embryo and larvae of fish are thought to be the life-cycle stage that is most sensitive to toxic effects (McKim et al., 1978). Short-term chronic assays are static-renewal tests developed by the EPA that commonly use fathead minnows (*Pimephales promelas*), *Daphnia magna,* or *Ceriodaphnia dubia* (small planktonic crustaceans) and a green algae (*Selenastrum capricornutum*) as test organisms (Birge et al., 1985; U.S. EPA, 1989). These assays examine growth (fathead minnow and algae), survival (minnow and *C. dubia*), and reproduction (*C. dubia*) after a 4- to 7-day exposure. The fathead minnow tests are basically truncated versions of the early-life-stage tests. However, *C. dubia* reach sexual maturity and begin to reproduce within a week of hatching, so the 7-day test for this species essentially encompasses the full life cycle.

Unlike tests on terrestrial organisms, where subjects are dosed with test chemicals via oral or inhalation routes, in aquatic toxicity tests the subjects are immersed in a solution of the contaminant. Therefore, the endpoints of aquatic toxicity tests are not recorded as LD_{50} or ED_{50} but as LC_{50} or EC_{50} (lethal and effective concentration). Results of the chronic tests are sometimes expressed as the maximum allowable toxicant concentration (MATC). This is a range of toxicant concentrations bounded by the *lowest observed effect concentration* (LOEC) at the upper end and the *no observable effect concentration* (NOEC) at the lower end. The LOEC and NOEC are determined by statistical analysis and are defined as the lowest toxicant concentration that elicits an effect that is statistically significantly different from the control and the highest concentration for which the effect is not significantly different from control, respectively (Mount and Stephan, 1967).

Toxicity tests have been used to measure toxic effects of individual chemicals or contaminated water collected from the field. Single-chemical tests are typically used for the purposes of chemical registry, while testing of contaminated water is commonly used for environmental monitoring purposes and to verify compliance with permitting requirements. In the latter case, water can be collected from the source of wastewater discharge ("effluent") or from the body of water receiving the effluent ("receiving water"). In these tests, the water to be tested is collected on site and test organisms are exposed to various concentrations diluted with clean water (ASTM, 1992c). Toxicity tests of undiluted effluent (referred to as *whole-effluent tests* or WETs; U.S. EPA, 1991a) are mandated by the Clean Water Act as part of the requirements for a permit to release effluents—a National Pollutant Discharge and Elimination System (NPDES) permit. Effluents are complex mixtures of multiple chemicals, some of which may contribute to the toxicity and some may not. Identification of toxic components of effluents may be facilitated by a process known as toxicity identification evaluation (TIE; U.S. EPA, 1993). In this process, different samples of the effluent are treated to remove various constituent chemicals (e.g., chelation to remove metals, extraction to remove organic contaminants) and each sample is tested. Reduction of the toxicity in any treated sample indicates that particular constituent has contributed to the toxicity of the whole effluent. TIEs are then followed by a Toxicity Reduction Evaluation (TRE). In this procedure, the source of the toxic constituents in the effluent may be

identified and possible methods to remove these toxic components from the effluent or reduce their toxicity are evaluated. This is followed by implementation of methods to control output of the toxic constituents or treat the effluent to reduce its toxicity. Follow-up toxicity testing is used to assess the efficacy of these remedial actions (U.S. EPA, 1991b).

Another type of test used for monitoring purposes is sediment toxicity testing. In this case, sediment is collected from the bottoms of lakes, rivers, bays, etc., and brought into the laboratory. Benthic invertebrates—commonly oligochaet worms, chironomid (midge fly) larvae, amphipod crustaceans, or mollusks—are then subjected to chronic or acute exposures and endpoints such as survival, growth, reproduction, and burrowing or other behaviors are recorded (ASTM, 1995). Important applications of sediment tests include determination of toxicity of sediments that are dredged from one location (e.g., in clearing shipping channels) and need to be disposed of at another location, environmental risk assessments of contaminated areas, and as a part of biomonitoring programs for the purpose of compliance to environmental regulations.

Sublethal Effects In the aquatic environment, concentrations of contaminants in the water or sediment may not be high enough to elicit mortality but may still induce sublethal effects on the health of aquatic organisms. One method by which these health effects can be determined is via histologic evidence of tissue damage or dysfunction (Teh et al., 1997). This type of damage can lead to diseases such as tumors or infectious and parasitic infestations. Tumor prevalence is commonly reported in bottom-dwelling fish and bivalve mollusks from contaminated areas (Van Beneden et al., 1993; Baumann, 1998; Wirgin and Waldman, 1998). Tumors are generally reported more often in feral populations of aquatic than terrestrial organisms. This is perhaps due to the less efficient DNA repair capacity of aquatic organisms (Wirgin and Waldman, 1998). Tumors are commonly reported in organisms that live within or in close proximity to the sediment, which is often highly contaminated with carcinogenic materials such as PAHs. Unlike cancers, infectious and parasitic diseases are not directly induced by contaminant exposure, but such exposure may increase the occurrence and severity of these infections. This is possibly due to suppression of leukocyte function (Chu and Hale, 1994; Couillard et al., 1995). The effects may be physically manifest as deterioration of the fins, skin lesions, or a high load of ecto- and endoparasites (Couillard et al., 1995; Landsberg et al., 1998). These detrimental effects on the health of aquatic organisms do not necessarily result in immediate mortality, but the life expectancy and relative reproductive output of the affected organisms may be compromised. If significant numbers of individuals are affected, this could ultimately have effects at the population level.

Field Studies Aquatic field studies can be classified as either manipulative or observational. In manipulative studies, previously unexposed organisms are used, and the experimenter determines the level of contamination to which they are exposed. In contrast, in observational studies, the level of contamination to which the organisms are exposed is not under the control of the experimenter. The objective of these studies may be collection of data for independent research projects or for monitoring organismal health and environmental quality as mandated by a regulatory authority. In the latter case, these studies are referred to as *biomonitoring*.

Aquatic field experiments in which treatments are applied by the experimenter include microcosms and mesocosms. The difference between microcosms and mesocoms is size. Microcosms are composed of large tanks, aquaria, or artificial pools. Mesocosms are artificially constructed ponds, plastic enclosures in lakes or ponds, or artificial streams. The attributes common to both are that they typically (but not always) contain more than one species of test organism, are located outdoors (although microcosms may also be located indoors), and are more complex than simple aquaria. They frequently contain sediment and/or vegetation or other structures and substrates that provide some degree of complexity and realism. The rationale is to produce a test system that contains some of the realism of the natural environment but is not so complex. Endpoints examined may include comparative acute toxicity (Stay and Jarvinen, 1995), biomarker expression (Eggens et al., 1996), or effects on aquatic populations and communities (Juettner et al., 1995; Barry and Logan, 1998). Although they are useful in providing information in some instances, their utility for regulatory purposes is controversial (Shaw and Kennedy, 1996).

Biomonitoring involves sampling aquatic organisms in the natural environment as an indication of the impact of anthropogenic contamination. Such activities may include confining test organisms in cages or sampling indigenous populations at contaminated sites. The relative advantages of using caged versus field-collected aquatic organisms are basically the same as those expounded in the preceding discussion on terrestrial enclosure studies. One endpoint that is particularly well suited to caging studies is acute and chronic toxicity. These endpoints are assessed by exposing caged organisms to contaminated water, sediment, or both and noting mortality and reproductive impairments. These types of tests are termed *ambient toxicity tests* (Stewart, 1996).

Evidence of overt toxicity is more difficult to determine in indigenous populations except during fish kills or other episodes of massive mortality. Consequently, endpoints other than mortality are more commonly documented in biomonitoring studies. One of the endpoints commonly measured is tissue concentrations of contaminants of concern (van der Oost, 1996a). These data are useful in determining whether chemicals present in the water or sediment are in a form that is bioavailable to aquatic organisms, for determining possible health risks to humans that might consume these organisms, or for modeling accumulation and effects in organisms at higher trophic levels. In addition, biomarkers or other sublethal effects may also be incorporated into aquatic biomonitoring programs (van der Oost et al., 1996b). This provides additional information as to whether the accumulated chemicals may be producing detrimental effects, assessing possible effects of complex mixtures and abiotic variables (e.g., water temperature) on toxic response. Additional endpoints used in biomonitoring may include effects on populations or communities (described above) or calculation of indices of water quality. One such index is known as the *Index of Biotic Integrity* (IBI). The variables used to calculate this index include percent pollution-tolerant species, percent of species from various trophic levels (e.g., herbivores, omnivores, top predators) or with various reproductive strategies, and occurrence of individuals with deformities, diseases, or other lesions (Karr, 1987). Each variable is given a numerical score that reflects its relative similarity to the reference sites. The scores are then summed to give a relative overall score for each sampling site. Analogous indices for benthic invertebrates are Hilsenhoff's biotic index and the Ephemeroptera-Plecoptera-Trichoptera (EPT) index, both of which rely on relative abundances of taxa that are thought to be pollution-tolerant and pollution-sensitive (Hoiland and Rabe, 1992).

A related idea in biomonitoring is the concept of indicator species. These can be species that are particularly tolerant or sensitive to environmental contamination such that their presence or absence is indicative of environmental degradation (Lang and Reymond, 1996). In this regard, indicator species may be used as a basis for biotic indices. For example, insects in the orders Ephemeroptera, Plecoptera, and Trichoptera are indicator species used in the EPT index. Alternatively, indicator species may be those in which biomarker responses to specific chemicals are well characterized, or species that are known to accumulate environmental contaminants. One such application is the Mussel Watch program (Wade et al., 1999) enacted by the United States National Oceanic and Atmospheric Administration (NOAA) in 1985. This program monitors contaminant tissue concentrations of coastal mussel populations as an indication of marine contamination. Indicator organisms such as these are useful in the detection of environmental contamination and its changes over time, and as early-warning indicators of possible ecologic effects.

The most efficacious methods of biomonitoring are those methods that integrate multiple endpoints at various levels of biological organization (e.g., chemical concentrations, biomarkers, community composition). For example, the *sediment quality triad* approach (Chapman, 1989) incorporates analysis of sediment chemical concentrations, acute toxicity, and benthic invertebrate community structure to assess the level of sediment contamination. Concordance between all three endpoints is taken as strong evidence that there are contaminants present in the sediment that could have detrimental effects on the aquatic ecosystem. There are analogous studies in fish that integrate endpoints at multiple levels of organization such as water and tissue chemical concentrations, biomarker expression, and population/community level effects (Adams et al., 1992). Integrated approaches such as these are necessary to evaluate environmental contamination in natural settings accurately, because the aquatic environment is too complex to be accurately assessed by one endpoint alone.

GOOD LABORATORY PRACTICES IN TERRESTRIAL AND AQUATIC ECOTOXICOLOGY

Good scientific practices, which result in high-quality data collection and interpretation, are of paramount importance in the field of toxicology. There is a great public demand for personal and environmental requirements under FIFRA and the Toxic Substances Control Act (TSCA), among other environmental legislation. With regulatory agencies increasingly being held accountable for environmental standards, there is a corresponding strong demand for formal and legal assurance that the toxicologic data generated are accurate and that sufficient documentation exists to support the study conclusions. Requirements are designed to ensure that the studies are conducted under high ethical and scientific standards. It is thus critical in today's regulated environment that toxicologic data are produced and reported in a manner that ensures the study is reconstructible and that there are sufficient assurances of the quality and integrity of the data.

The principles and practices of quality assurance and quality control are perhaps best exemplified by the Good Laboratory Practice Guidelines (GLPs). The GLPs are regulation standards that define conditions under which a toxicology study should be planned, conducted, monitored, reported, and archived. They have been adopted by many national and international governments and agencies. The GLPs outline study management procedures and documentation practices that, if followed, will limit the influence of extraneous factors on study results and interpretations. GLPs include provisions for such factors as personnel management and training, facilities support and operation, equipment design, maintenance and calibration, independent quality assurance monitoring, handling of test systems and materials, documentation of study conduct, written standard operation procedures and study protocols, reporting study results, and retention of records and samples.

The U.S. EPA implemented GLP regulations in 1983 under the mandate of FIFRA (40 CFR Part 160) and TSCA (40 CFR Part 792) for pesticide and toxic chemical registration and use. By 1989, these regulations were amended to cover field studies as well as laboratory studies. Today, significant efforts are under way to provide international harmonization of the regulations/standards in the field of ecotoxicology.

MODELING AND GEOGRAPHIC INFORMATION SYSTEMS

Modeling in ecotoxicology allows the prediction of effects of toxic compounds on the environment, which can be characterized by various ecosystems. Terrestrial ecosystems include forests, grasslands, and agricultural areas, whereas aquatic ecosystems include lakes, rivers, and wetlands. Each of these systems is a collection of interconnected components, or subsystems, that functions as a complete entity. Because the dynamics of real systems are quite complex, an understanding of the impacts of toxicants on a system can be enhanced by modeling that system.

The components, or compartments, of a system are represented by state variables that define the system. Once we have defined the system, it is possible to identify stimuli or disturbances from exogenous toxic substances, called *inputs,* from outside the system. These inputs operate on the system to produce a response called the *output.* The adverse effects of many toxic inputs are directly related to their ability to interfere with the normal functioning of both physiologic and environmental systems. For example, emissions of heavy metals from a lead ore-processing complex caused perturbations to the litter-arthropod food chain in a forest ecosystem (Watson, et al., 1976). Elevated concentrations of lead (Pb), zinc (Zn), copper (Cu), and cadmium (Cd) caused reduced arthropod density and microbial activity, resulting in a lowered rate of decomposition and a disturbance of forest nutrient dynamics.

In applying modeling to ecotoxicology, we are interested in studying a "real world" system and the effects of various toxicants on that system. A model is a necessary abstraction of the real system. The level of abstraction, however, is determined by the objectives of the model. Our objective is to stimulate the behavior of a system perturbed by a toxicant. This requires a mechanistic approach to modeling.

The modeling process involves three steps: (1) identification of system components and boundaries, (2) identification of component interactions, and (3) characterization of those interactions using quantitative abstractions of mechanistic processes. Once the model has been defined, it is implemented on a computer. Measurements obtained from the real system are compared with the model projections in a process of model validation. Improvements are then made to the model by changing parameter values or modifying equations in the model. Several iterations of comparing

A related idea in biomonitoring is the concept of indicator species. These can be species that are particularly tolerant or sensitive to environmental contamination such that their presence or absence is indicative of environmental degradation (Lang and Reymond, 1996). In this regard, indicator species may be used as a basis for biotic indices. For example, insects in the orders Ephemeroptera, Plecoptera, and Trichoptera are indicator species used in the EPT index. Alternatively, indicator species may be those in which biomarker responses to specific chemicals are well characterized, or species that are known to accumulate environmental contaminants. One such application is the Mussel Watch program (Wade et al., 1999) enacted by the United States National Oceanic and Atmospheric Administration (NOAA) in 1985. This program monitors contaminant tissue concentrations of coastal mussel populations as an indication of marine contamination. Indicator organisms such as these are useful in the detection of environmental contamination and its changes over time, and as early-warning indicators of possible ecologic effects.

The most efficacious methods of biomonitoring are those methods that integrate multiple endpoints at various levels of biological organization (e.g., chemical concentrations, biomarkers, community composition). For example, the *sediment quality triad* approach (Chapman, 1989) incorporates analysis of sediment chemical concentrations, acute toxicity, and benthic invertebrate community structure to assess the level of sediment contamination. Concordance between all three endpoints is taken as strong evidence that there are contaminants present in the sediment that could have detrimental effects on the aquatic ecosystem. There are analogous studies in fish that integrate endpoints at multiple levels of organization such as water and tissue chemical concentrations, biomarker expression, and population/community level effects (Adams et al., 1992). Integrated approaches such as these are necessary to evaluate environmental contamination in natural settings accurately, because the aquatic environment is too complex to be accurately assessed by one endpoint alone.

GOOD LABORATORY PRACTICES IN TERRESTRIAL AND AQUATIC ECOTOXICOLOGY

Good scientific practices, which result in high-quality data collection and interpretation, are of paramount importance in the field of toxicology. There is a great public demand for personal and environmental requirements under FIFRA and the Toxic Substances Control Act (TSCA), among other environmental legislation. With regulatory agencies increasingly being held accountable for environmental standards, there is a corresponding strong demand for formal and legal assurance that the toxicologic data generated are accurate and that sufficient documentation exists to support the study conclusions. Requirements are designed to ensure that the studies are conducted under high ethical and scientific standards. It is thus critical in today's regulated environment that toxicologic data are produced and reported in a manner that ensures the study is reconstructible and that there are sufficient assurances of the quality and integrity of the data.

The principles and practices of quality assurance and quality control are perhaps best exemplified by the Good Laboratory Practice Guidelines (GLPs). The GLPs are regulation standards that define conditions under which a toxicology study should be planned, conducted, monitored, reported, and archived. They have been adopted by many national and international governments and agencies. The GLPs outline study management procedures and documentation practices that, if followed, will limit the influence of extraneous factors on study results and interpretations. GLPs include provisions for such factors as personnel management and training, facilities support and operation, equipment design, maintenance and calibration, independent quality assurance monitoring, handling of test systems and materials, documentation of study conduct, written standard operation procedures and study protocols, reporting study results, and retention of records and samples.

The U.S. EPA implemented GLP regulations in 1983 under the mandate of FIFRA (40 CFR Part 160) and TSCA (40 CFR Part 792) for pesticide and toxic chemical registration and use. By 1989, these regulations were amended to cover field studies as well as laboratory studies. Today, significant efforts are under way to provide international harmonization of the regulations/standards in the field of ecotoxicology.

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model behavior to that of the real system are usually required to obtain a satisfactory or "valid" model.

Types of Models

Models can be used to obtain qualitative or quantitative information about a complex system. Qualitative models emphasize the relationship between the variables of interest while minimizing the requirement for tremendous accuracy in the parameters of the model. The disadvantage is that there can be no reliance on the numbers produced from such a model. Quantitative ecotoxicologic models can be classified as (1) individual-based versus aggregated models, (2) stochastic versus deterministic models, and (3) spatially distributed versus lumped models. Such models often have hundreds of thousands of lines of code and are used only at the final stages of a system simulation. The results of such simulations are always regarded with suspicion until they are verified by actual data obtained in real systems. The development of such models usually takes place over a number of years as opposed to hours or days for models that are used for qualitative information.

Individual-Based versus Aggregated Models Models that stimulate all individuals simultaneously are referred to as *individual-based models* (e.g., Huston et al., 1988; DeAngelis and Gross, 1992). Each individual in the simulation has a unique set of characteristics: age, size, condition, social status, and location in the landscape as well as its own history of daily foraging, reproduction, and mortality. The individual-based approach has several advantages. It enables the modeler to include complex behavior and decision making by individual organisms in the model. But importantly, it allows one to model populations in complex landscapes, where different individuals may be exposed to very different levels of toxicant concentration (DeAngelis, 1994).

Models of individuals can be extended to a population as a whole by (1) simulating not just one individual but all individuals that make up the population of interest or (2) aggregating various population members into classes, such as age classes. *Aggregated models,* then, follow not individual organisms but variables representing the numbers of individuals per age class. In simulating a complex environmental system, both individual-based and aggregated models will be needed. Usually individual-based models are used to represent vertebrate species while aggregated models are used to represent organisms at lower classification levels.

Stochastic versus Deterministic Models Model coefficients can be functions not only of other variables but also of random variables; thus they can be random variables themselves. In this way of classifying models, those with random (stochastic) variables are called *stochastic models* and those without are called *deterministic models.* Random variables are used to represent the random variation or "unexplained" variation in the state variables. Stochastic models also can include random variables expressed either as random inputs or as parameters with a random error term.

In a stochastic model, random variables representing state variables, model parameters, or both will take on values according to some statistical distribution. In other words, there will be a probability associated with the value of the parameter or state variable.

Monte Carlo is a numerical technique of finding a solution to a stochastic model. For those random features of the model, values are chosen from a probability distribution. Repeated runs of the model then will result in different outcomes. A probability distribution can be calculated for a state variable in the model along with its mean and variance. Suppose the model has random variables for parameters $p_1, p_2, p_3, \ldots p_n$; the state variable will then be a function of the n parameters. A value for each parameter is calculated by sampling from its individual distribution function. A value for the state variable X then is obtained by running a simulation of the model. We repeat the process until we have N values of the state variable X. Finally we determine the mean (μ) and variance (σ^2) for X.

Spatially Distributed versus Lumped Models Lumped models spatially integrate the entire area being modeled (Moore et al., 1993). Parameters for lumped models are averaged over the same spatial area. Spatially distributed models are based upon identifiable geographic units within the area being modeled. These subunits can represent physiographic areas, such as hydrologic or atmospheric basins, which can be identified using a geographic information systems (GIS). A GIS can be used to further identify homogeneous polygons or grid cells based upon soil and terrain features. Model parameters for each subunit can be geographically referenced and stored in the GIS database. The distributed model can then be used to simulate the response to a spatially distributed toxicant by replicating the model for each geographic subunit. Responses to toxicants are likely to be spatially nonlinear. Therefore, a lumped model using mean parameter values will not yield the expected value of the combined results of a distributed model. Modeling in ecotoxicology usually will involve individual-based, stochastic, spatially distributed models.

Modeling Exposure

Exposure of organisms to toxicants requires contact between organisms and the toxicant of concern. Modeling exposure requires a model that will predict the spatial and temporal distributions of the toxicant and a model that will predict the organism's geographic position relative to the toxicant concentration. Transport and fate models are used to predict the spatial distribution of toxicants. Atmospheric transport models (for example, CALPUFF (U.S. EPA, 1995a) and ISC3 (U.S. EPA, 1995b) predict ground-level concentrations of toxicants from stack emissions. Dixon and Murphy (1979) used an atmospheric transport model to predict exposure concentrations as a series of "plume events" at any point on the ground (Fig. 29-2*A*). Exposure can occur from inhalation, immersion, ingestion, or a combination of these. Some vegetation models CERES (Dixon et al., 1978a,b, Luxmoore et al., 1978) and PLANTX (Trapp et al., 1994) can predict uptake of atmospheric and soil concentrations of toxicants. Surface hydrologic models HSPF (Donigian et al., 1983) and GLEAMS (Leonard et al., 1987) predict the runoff of toxicants from the land surface. Lake and stream models obtain input from surface runoff models and predict the change in toxicant concentration within the water body.

Most vertebrates are mobile enough to move from an area of high toxicant concentration to an area of low toxicant concentration (or vice versa). The actual exposure of an animal will depend upon the concentration levels at the geographic locations visited by the animal at the time of the visit. An integrated time- and space-averaged exposure E_i can be calculated using the model (Ott et al., 1986; Henriques and Dixon, 1996):

$$E_i = \sum_{j=1}^{J} c_j t_{ij} \tag{1}$$

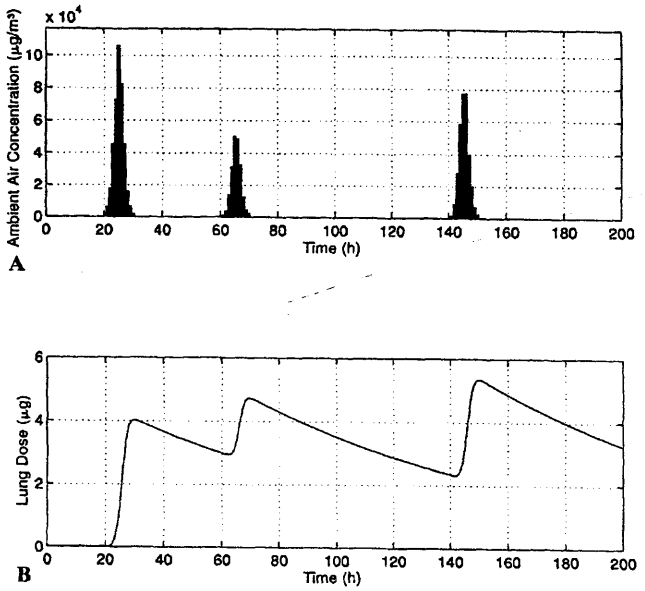


Figure 29-2. **(A).** ***Ambient air concentrations of toxicant predicted by discrete-event gaussian plume model over time. Each pulse represents the ground-level concentration at a given location. The shape of the pulse depends upon wind direction, wind speed, and atmospheric stability.*** **(B).** ***Lung dose of toxicant predicted from the lung model [Eq. (2)] with input from the plume model. The dose increases as plume passes over animal's location. The dose decreases as plume changes direction and concentration in lung decreases by diffusion to the bloodstream.***

where c_j = exposure concentration in microenvironment j
t_{ij} = time spent by animal i in microenvironment j
J = total number of microenvironments occupied by animal i

The prediction of real-time exposure requires linking transport models with behavioral models of animal movement (Sathe, 1997). Models of animal movement can be based upon matching spatial patterns of observed behavior (Siniff and Jessen, 1969), rules based upon mechanisms governing the response of an individual to its environment (Wolff, 1994), or theoretical constructs such as random-walk models (Holgate, 1971; Tyler and Rose, 1994).

Modeling Effects

The effect of a toxicant on an organism depends upon the dose (the concentration reaching the target organ) and the physiologic response to the dose. The dose depends upon the concentration of the chemical at the exposure site and the duration of the exposure. To predict the concentration reaching the target organ, we need to know how much of the chemical is taken up and absorbed by the organism. We also need to know where the chemical is distributed among the organism's tissues and organs, and the rate at which the chemical is excreted from the same tissues and organs.

The dynamics of the disposition of a toxicant in the body of an organism is the subject of toxicokinetics. The dynamics involve the concentration changes over time of a toxicant in various tissues and the rate processes that control the movement from one part of the organism to another. For example, the dynamics of a toxic gas or vapor in the lungs, dC/dt (µg/h), can be simulated with the model:

$$\frac{dC}{dt} = 10^{-6}\, Y \cdot V_T \cdot f - k \cdot C \qquad (2)$$

where Y = exposure concentration (µg/m^3)
V_T = tidal volume (milliliters per breath)
f = breathing frequency (breaths per hour)
k = transfer rate from lungs to bloodstream (L/h).

A solution to Eq. (2), with the input from the discrete-event atmospheric exposure model, can be obtained by integrating over a defined time period and initial concentration (Fig. 29-2*B*).

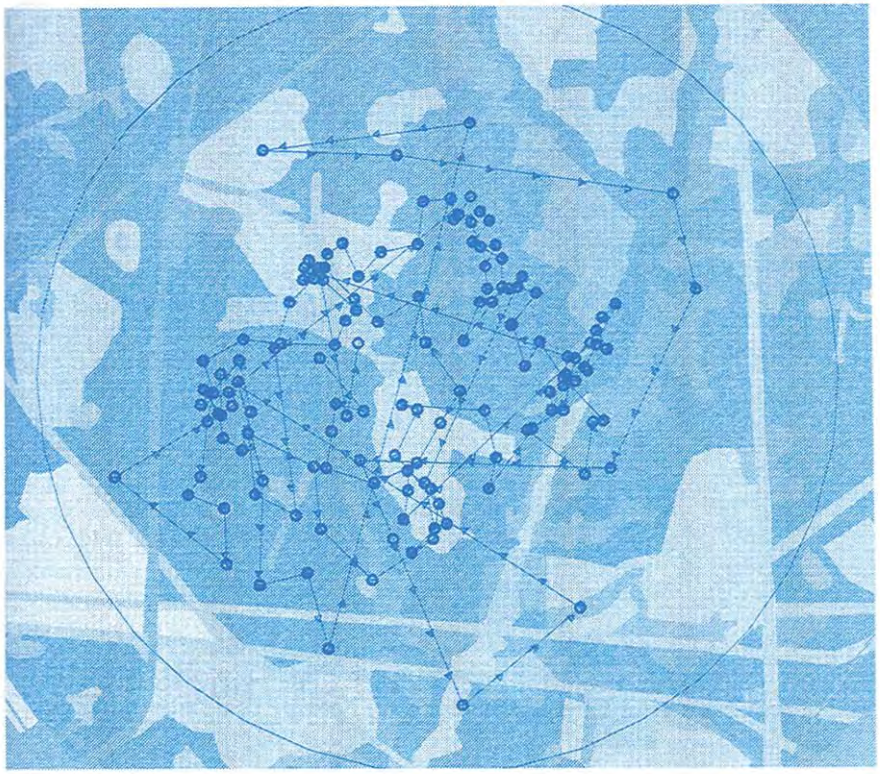
Linking Models to Geographic Information Systems

Geographic information systems (GISs) can be used to map the observed and predicted concentrations of toxic substances as well as the resulting effects of exposure to these concentrations. By linking models with GISs, the ability to explicitly model spatial dynamics of toxicant concentrations is greatly enhanced. There are different levels of integration of models with a GIS. First, a set of utility programs, external to both the model and the GIS, can be used to transfer data between the model and the GIS. Second, routines and macros can be written in the GIS language to run the models and analyze the results. And third, the GIS computer code can be modified to run the models and display the results of the simulations as part of the GIS procedures.

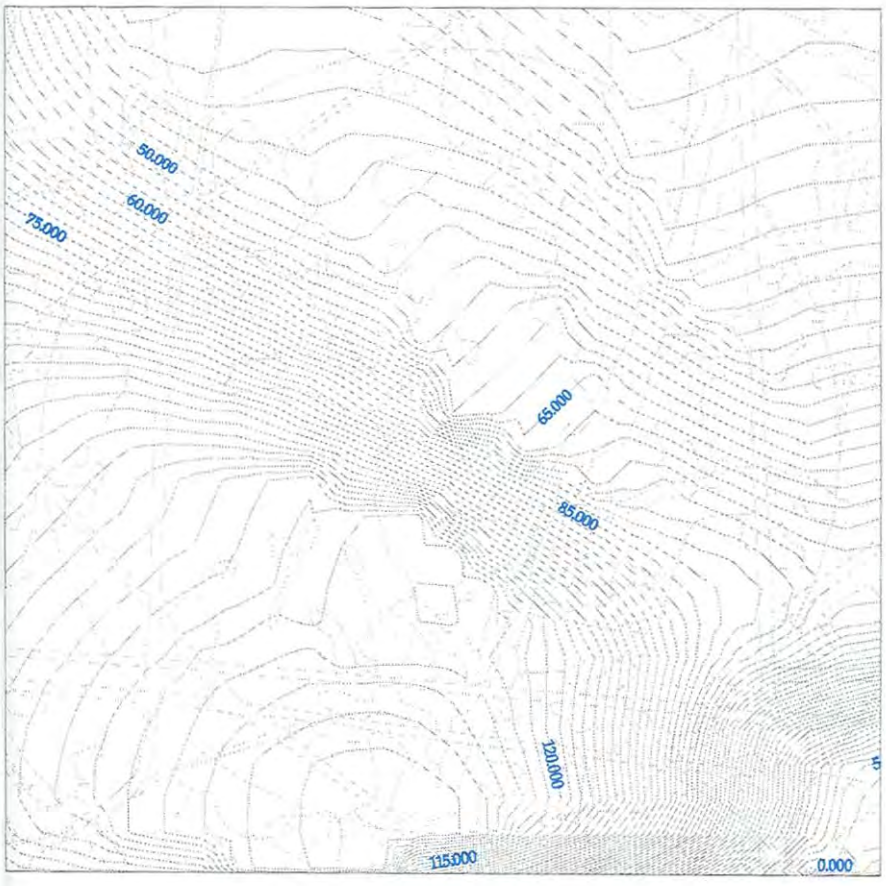
Mapping Exposure and Effects Results from simulation models with spatially referenced output can be mapped as static or dynamic data. Static data are spatially explicit but are expressed as a point (snapshot), an average, or a summed response over time. Dynamic data consider responses of state variables at points in space or a sum of the responses for an area and can be graphed as a function of time, or time series.

In our lung-model example, static exposure can be mapped using the spatial behavior model (Fig. 29-3*A*) and the discrete-event gaussian plume model (Fig. 29-3*B*). The resulting effect (lung dose) also can be mapped spatially (Fig. 29-3*C*). The results from the discrete-event plume model and the lung model can be graphed as a time series (Fig. 29-2*A*,*B*). This response is a result of the animal moving in space and time and the different concentrations of toxicant to which it is exposed at those places and times. A population response can be predicted by repeating the procedure for all the individuals in the exposed population. A spatial map of the steady-state lung concentrations in the population (Fig. 29-4*A*) shows a static response. The population response can also be expressed as a probability distribution (Fig. 29-4*B*) to show the variability in the population that results from the different individual responses.

Displaying both spatial and temporal responses simultaneously is more difficult, although recent developments in the area of computer visualization make this possible. Dynamic shifts in spatial maps of animal behavior (home range) and ambient concentrations can be illustrated in a "movie" sequence of maps. It is also possible to use visualization methods to show "real time" movement of an animal in space and simultaneously display the time-series graph of lung dose. These techniques can enhance our understanding of the effects of toxicants in the environment and provide for more realistic estimates of risk to those toxicants.



A



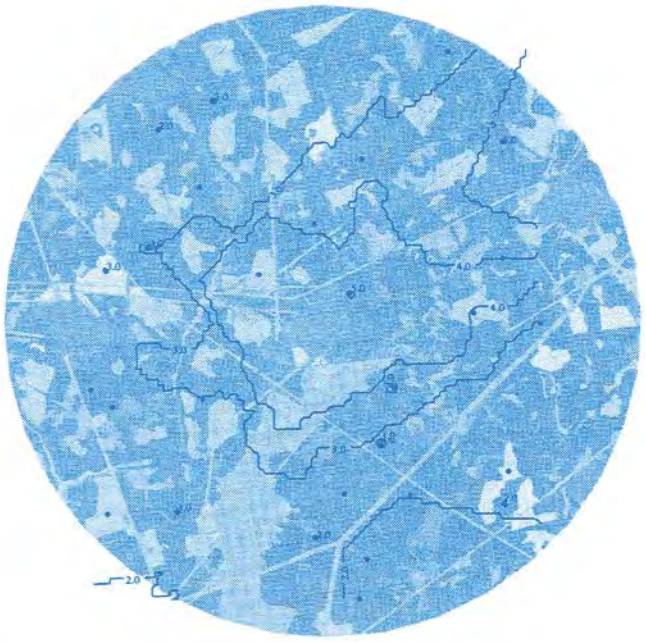
B

***Figure 29-3. Example of an animal's movement predicted by a spatial behavior model* (A). *Example of static ambient ground-level concentrations of a toxicant predicted by Gaussian plume model* (B).**

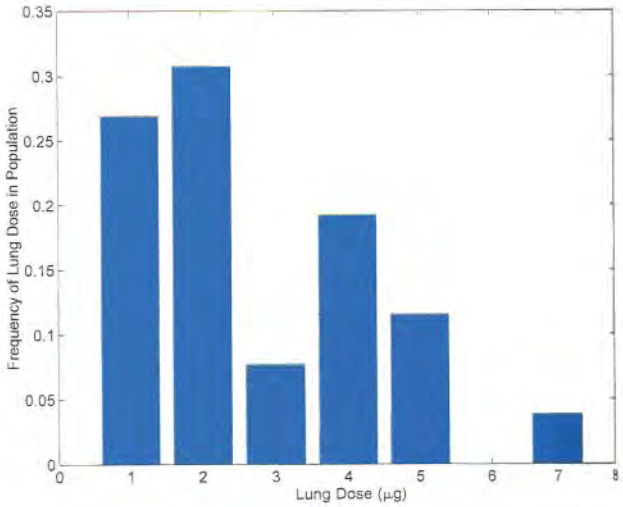
Isopleths show lines of equal concentration (units are in micrograms per cubic meter.) (From Henriques and Dixon, 1996.) Predicted dose to the lungs from exposure to ambient toxicant concentration as the animal moves according to the pattern in *(A)*. Dose depends upon the exposure concentration *(B)* and the time the animal spends at each location in *(A)*. Isopleths are lines of equal lung dose in units of μg *(C)*. Predicted dose to the lungs from exposure to ambient toxicant concentration as the animal moves according to the pattern in *(A)* depends upon the exposure concentration *(B)* and the time the animal spends at each location in *(A)*. Isopleths are lines of equal lung dose in units of micrograms.

C

Figure 29-3. ***(Continued)***



A



B

Figure 29-4. **(A).** ***Predicted static population lung dose for 26 individual animals. Dose is the maximum for that individual and mapped at the center of the animal's home range. Individual in Fig. 29-2A is found in the northwest quadrant.*** **(B).** ***Frequency of occurrence of lung dose in population mapped in*** **(A).**

ECOLOGIC RISK ASSESSMENT

With the growth of environmental toxicology comes the need to appropriately assess and quantify the impact of toxic chemicals on organisms, their populations, and communities in ecosystems. Earlier techniques to conduct risk assessments utilizing human health approaches were not appropriate for ecologic systems. For this reason, the U.S. EPA issued a framework for conducting ecologic risk assessment (U.S. EPA, 1992a). This framework, which was expanded and modified in 1998 (U.S. EPA, 1998), allows for the assessment of the impact of toxic chemicals as well as other stressors on ecologic systems (Fig. 29-5). In the problem-formulation phase, the potential pathways and species that might be affected by the toxic substance are considered. As part of the problem-formulation phase, a conceptual model is usually developed describing routes of exposure, biota of concern, and anticipated effect endpoints. The actual risk of chemicals to wildlife or other biota is then determined using exposure data and toxic effects of the chemicals of interest (Table 29-2 demonstrates for agriculture chemicals). Toxicity data for species of concern at either the individual or population level are also incorporated (Kendall and Akerman, 1992). In the risk-characterization phase, exposure and effect data accumulated in the analysis phase are combined and the risk potential is characterized. Based on the resulting risk, risk-management steps can be taken, generally involving decreasing the exposure portion of the assessment, in order to decrease the overall risk.

One example involving the assessment of the ecologic risk to wildlife of exposure to the insecticide carbofuran (a carbamate) has been published by the U.S. EPA (Houseknecht, 1993). Ecologic risk assessment revealed widespread and repeated mortality events, particularly in locations where birds ingested carbofuran granules in agricultural ecosystems. According to legislation promulgated by FIFRA and extended to the international sphere by the Migratory Bird Treaty Act, environmental regulations do not permit the killing of migratory songbirds or waterfowl with a pesticide. Under FIFRA, through a special review, the U.S. EPA took regulatory action against a carbamate, carbofuran (2,3-Dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate), used in a large number of agroecosystems in which such use was associated with wildlife mortality.

Under the U.S. EPA's risk-assessment paradigm, risk characterization offers the opportunity to put the ecologic risk in per-

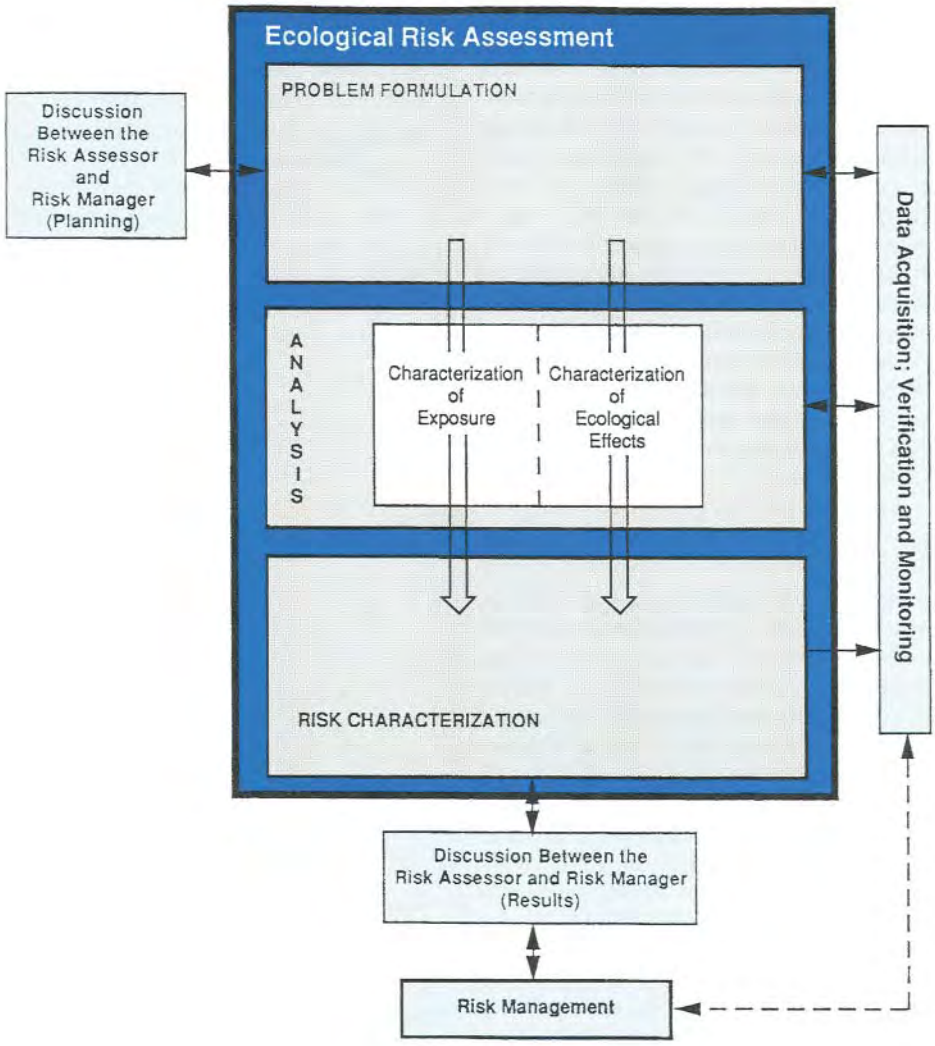


Figure 29-5. Generalized framework for ecologic risk assessment. (From U.S. EPA, 1998.)

Table 29-2
Ecotoxicological Assessment Criteria for Pesticides

PRESUMPTION OF MINIMUM HAZARD	HAZARD THAT MAY BE MITIGATED BY RESTRICTED USE	LEVEL OF CONCERN (LOC)
Mammals and Birds	Granular Formulations	
LD_{50}/sq. ft. $< \frac{1}{5} LD_{50}$	$\frac{1}{5} \leq LD_{50}$/sq. ft. $< \frac{1}{2} LD_{50}$ $LD_{50} \geq 50$mg/kg	LD_{50}/sq. ft. $> \frac{1}{2} LD_{50}$
	Acute Toxicity	
EEC* $< \frac{1}{5} LC_{50}$	$\frac{1}{5} LC_{50} \leq EEC < \frac{1}{2} LC_{50}$	EEC $\geq \frac{1}{2} LC_{50}$
mg/kg/day $< \frac{1}{5} LD_{50}$	$\frac{1}{5} LD_{50} \leq$ mg/kg/day $< \frac{1}{2} LD_{50}$	mg/kg/day $\geq \frac{1}{2} LD_{50}$
Aquatic Organisms		
EEC $< \frac{1}{10} LC_{50}$	$\frac{1}{10} LC_{50} \leq EEC < \frac{1}{2} LC_{50}$	EEC $\geq \frac{1}{2} LC_{50}$
Mammals, Birds, and Aquatics	Chronic Toxicity	
EEC < Chronic No effect level	N/A	EEC ≥ effect level (including reproductive)

SOURCE: EPA guidelines provided by Edward Fite, Office of Pesticide Programs, Ecological Effects Branch, EPA Headquarters, Washington, DC. From: *Wildlife Toxicology and Population Modeling: Integrated Studies of Agroecosystem.* Boca Raton, FL: CRC/Lewis, 1994, with permission.
*Estimated environmental concentration. This is typically calculated using a series of simple nomographs to complex exposure models.

spective and to identify uncertainty in the development of the risk assessment. Although carbofuran could not be proven to cause significant adverse effects on bird populations, widespread and repeated mortality was evident and regulatory action was taken (Houseknecht, 1993; U.S. EPA, 1989). Evidence of carbofuran killing bald eagles (*Haliaeetus leucocephalus*) added to the overall concern for this chemical. Under the auspices of the Endangered Species Act, endangered species in the United States required special consideration because of their limited numbers and possible susceptibility to extinction.

The quotient method of assessing risk is often utilized in ecologic risk assessment (Bascietto et al., 1990). The quotient method employs the formula of the expected environmental concentration divided by the toxic impact of concern (e.g., LC_{50} or EC_{50}). If the quotient exceeds 1, then a significant risk may be indicated. Indeed, granular carbofuran products utilized in a broad range of agricultural uses resulted in quotients exceeding 1, and, as mentioned earlier, wildlife mortality was identified (Houseknecht, 1993).

Probabilistic Risk Assessment

Ecologic risk assessment continues to evolve as a science (Suter, 1993). Probabilistic risk assessments are used to further refine risk assessments so that they reflect actual risk in the environment. Probabilistic risk assessments have been used for several years in other disciplines such as predicting accidents, systems failure, and weather forecasting, but they have been used in ecologic risk assessments only recently. Probabilistic risk assessments can range from the use of probability distributions in place of point estimates in the Quotient Method to overlapping distributions of exposure and toxicity to stochastic simulation models.

Overlapping Distributions Overlapping probability distributions have been described in detail (Cardwell et al., 1993; SETAC, 1994; Parkhurst et al., 1995) and have been used in a number of ecologic risk assessments (Solomon et al., 1996; Giesy et al., 1999). In this approach, cumulative frequencies of environmental exposure concentrations (EECs, generally in milligrams per kilogram on food items) and toxicity values (LC_{50}, LC_{10}, or LC_5 transformed to a value of milligrams per kilogram per day) are plotted on the same graph. Frequencies are plotted on the *Y* axis using a probability scale and the concentrations plotted on the *X* axis using a logarithmic scale (Fig. 29-6). Toxicity values are ranked in as-

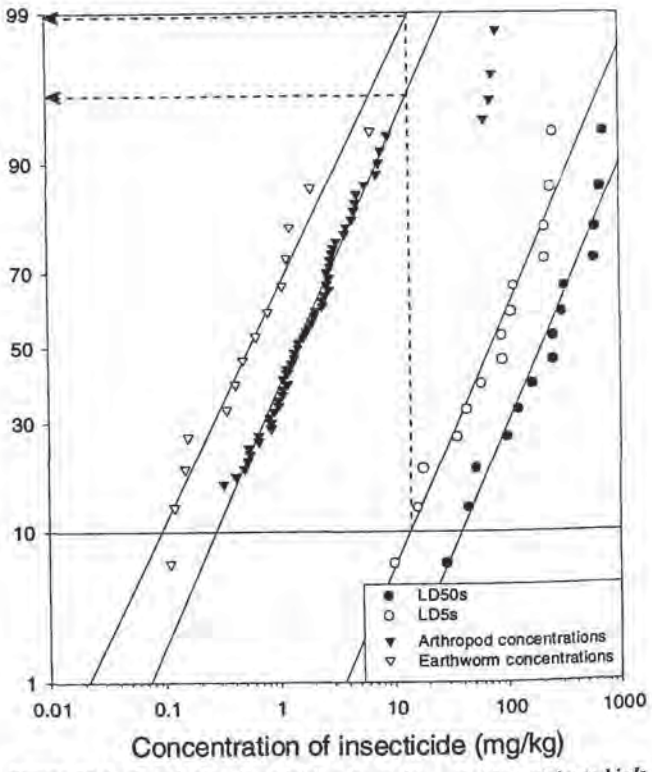


Figure 29-6. Graph showing distributions of insecticide toxicity to birds (expressed as concentration in food items) compared to actual concentrations reported in collected food items.

Comparing the distributions, the 10th centile of avian toxicity (LD_5 values) would be exceeded 1 percent of the time for earthworm consumption and 5 percent of the time for other invertebrates.

cending order and then transformed to cumulative percentages using the transformation:

$$\frac{100 \times i}{n + 1}$$

where i = ith observation of a total of n observations, starting with the lowest toxicity value. The resulting plots show an approximate linear relationship between frequency and the exposure and toxicity data, and linear regression can be used to fit straight lines to the data. The area of overlap between the two lines (if any) then can indicate the level of risk to the organisms exposed to the EECs. In the example shown in Fig. 29-6, exposure concentrations are the residues from the insecticide chlorpyrifos found on arthropods and earthworms. These data were ranked in the same way as the toxicity data. In this example, there is very little overlap of the two distributions. Using the 10th centile as an exceedance level, the LD_5s would exceed this level about 1 percent of the time for consumption of arthropods and about 5 percent of the time for consumption of earthworms (ECOFRAM, 1999).

Stochastic Simulation In probabilistic risk assessments using simulation, probability distributions are measured (or estimated) for parameters to account for natural variation, lack of knowledge, or uncertainty. The actual parameter values used in a simulation are obtained by sampling their distributions in a Monte Carlo process. The resulting model output will contain endpoint values, one value for each set of parameter values in a given simulation (see "Modeling and Geographic Information Systems," above). Several simulations will yield a probability distribution of endpoint values, such as mortality percentage in the simulated population. By altering the mean value of the model parameters and running additional sets of simulations, the percentage of outcomes that exceed a certain level of mortality can be estimated (Fig. 29-7). This curve can be compared with a graph of the "threshold of acceptability" defined by the risk manager to determine whether there is the potential for unacceptable risk.

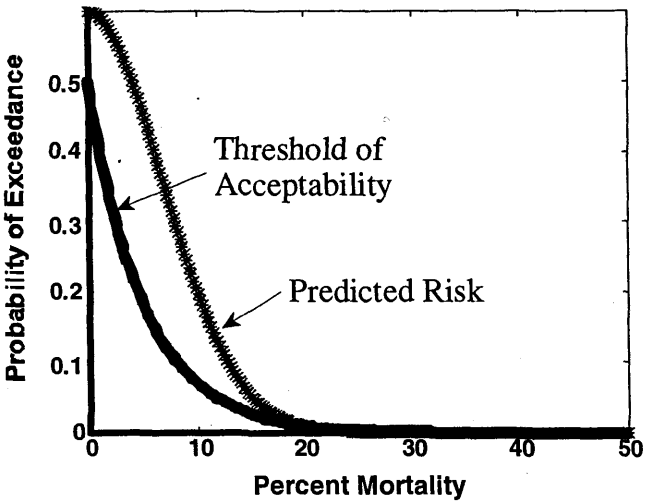


Figure 29-7. Illustration of risk manager's threshold of acceptability (shaded line) and predicted risk from a simulation model (data points).

A comparison of the two curves shows an area where the predicted risk exceeds the acceptability threshold, indicating a potentially unacceptable risk. (Adapted from ECOFRAM, 1999.)

Examples of probabilistic ecologic risk assessments include the effects of the herbicide atrazine [6-chloro-*N*-ethyl-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] on aquatic ecosystems in the midwestern U.S. corn belt (Solomon et al., 1996) and the insecticide chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) ester] on aquatic ecosystems (Giesy et al., 1999), also in the midwestern United States.

The key to understanding ecologic risk assessment in ecotoxicology is considering more than just chemical toxicity. We must consider ecologic risk assessment in the context of exposure and other issues such as sublethal effects or ecosystem impacts. Indeed, we now know that predator-prey relationships can be affected by chemical exposure in prey (Galindo et al., 1985). In addition, "biomarkers" offer new technologies to assess sublethal impacts of chemicals on fish and wildlife populations (Dickerson et al., 1994).

The availability of data from laboratory and field ecotoxicologic experiments generated under GLPs, as discussed above, will improve the quality and ultimate value of ecologic risk assessments. In probabilistic risk assessments, the amount of data required increases substantially as the point estimates for toxicity and exposure are replaced by distributions and model parameters with error terms. Good Laboratory Practices data may offer new opportunities to integrate validated information into ecologic effect or exposure models for use in risk assessment (Kendall and Lacher, 1994). The contribution of ecologic models in the ecologic risk assessment process is in its infancy and offers significant opportunities for the extrapolation of data from laboratory and field experiments to a broader range of applications for the protection of the environment and its fish, wildlife, and other biotic resources.

ENVIRONMENTAL TOXICOLOGY AND HUMAN HEALTH

Links between wildlife and human health serve as a premise for extrapolation in risk assessment. Humans share many cellular and subcellular mechanisms with wildlife species. Humans and wildlife also overlap in their physical environment and therefore are exposed to many of the same contaminants. There is evidence to suggest that when highly conserved systems are targeted by environmental toxicants, both ecosystem and human health suffer.

There are obvious challenges and concerns in the extrapolation of wildlife data to humans. When there are contaminant-specific alterations in wildlife health, concerns about coordinate adverse effects in humans tend to focus on susceptible developmental periods, including in utero, neonatal, pubertal, lactational, and menopausal stages (Colburn et al., 1993). There is also a real concern about an increased risk of various cancers caused by environmental contaminants (Kavlock et al., 1996) and populations with genetic or environmental susceptibility (Frame et al., 1998). The overall rate of some cancers is increasing, particularly in industrialized countries. Based on animal models, chemical exposure figures in the etiology of many cancers; therefore a link to human cancer incidence seems plausible. Unfortunately, linking known contaminant exposures to an affected human population is difficult, particularly when effects are not identified for many years. By the time human effects are identified, the causative agent may not be present or detectable.

As with wildlife, some human health effects may be reversible while others may involve irreversible changes. In some instances, this may be a matter of dose. A high dose may lead to irreversible

direct effects, such as malformations. However, low doses may manifest as subtle or latent functional changes in susceptibility that are not apparent until after the exposure has passed and the individual is "challenged." Particularly because of the longevity of humans, even low-dose exposures may result in a human health risk, predisposing elderly individuals to chronic disease processes. Wildlife may not be affected in the same ways because of their generally shorter life span.

Regardless of species, the process of risk assessment requires four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Often, these processes are difficult in human populations and extrapolations are required, including qualitative interspecies extrapolation from test animal to human and quantitative extrapolation from high to low dose. Uncertainties in these two extrapolations have sometimes resulted in a low confidence in risk estimates for humans. When human data are of low quality or not available, wildlife sentinels can serve a useful role in assessing human risk. For the future, however, much more information is needed to develop the human database regarding exposure, susceptibility, metabolism and disposition, site and mechanisms, tissue repair processes, compensatory responses, and adaptive mechanisms. Obviously, the more human data available for risk assessment, the better, and the more generalized and relevant to real human health effects, the easier it is to define a risk-management strategy (Smith and Wright, 1996).

Thus far, the best wildlife-to-human extrapolations have relied on strong, consistent human data available from high-dose accidental exposures for comparison with wildlife effects from monitoring studies; the Yusho and Yu-Cheng PCB incidents (Masuda et al., 1979; Kuratsune et al., 1976; Hsu, 1985); the TCDD accident in Seveso, Italy (Mocarelli et al., 1991); and human exposure to diethylstilbestrol (DES). The dose-response data collected, the large numbers of affected individuals, and an understanding of biological mechanisms in each of these cases make the comparisons possible. For most low-dose exposures, the ability to show causation is still poor. Future research relating the environmental health problems of wildlife and humans should recognize the scope of environmental disease processes and species-specific endpoints that reflect the divergence as well as the conservation of systems.

The interconnections between ecologic health and human health should not be overlooked. The indirect effects of environmental pollution may, in the end, be more important than the direct effects for human health. The environment is thought to act as a buffer for both toxicants and disease. However, even a buffer has its limits. For instance, the human population is at greater risk for emerging diseases as the natural environment dwindles in relative area. In the future, it is important that researchers focus on closing the artificial gap that views "environmental" or "human" health issues separately.

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