chapter 30

Ecotoxicology

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INTRODUCTION

Ecotoxicology is the science of contaminants in the biosphere and their effects on constituents of the biosphere (Newman and Unger, 2003). It follows from this definition that ecotoxicologists examine large-scale ecological phenomenon (Preston, 2002) in addition to those normally addressed in toxicology; ecotoxicology has an overarching goal of explaining and predicting effect or exposure phenomena at several levels of biological organization (Fig. 30-1). Essential explanations and models include those applied in conventional toxicology and a range of environmental sciences.

Although Truhaut's original definition of this new science encompassed effects to humans (Truhaut, 1977), most recent definitions of ecotoxicology do not. Relevant effects to nonhuman targets range from biomolecular to global. Taking on the classic toxicology vantage initially, suborganismal and organismal effects were emphasized during ecotoxicology's nascent stage; however, studies of higher level effects and interactions are becoming increasingly commonplace as the science matures. Such indirect effects[1] were initially considered problematic and reluctantly relegated to secondary importance (Fleeger *et al.*, 2003) relative to direct effects to individuals. Indirect effects are now known to be as important as direct effects to nonhuman targets (Fleeger *et al.*, 2003; Chapman, 2004). As the need to predict major effects to populations, communities, ecosystems, and other higher level entities has become increasingly apparent, more cause–effect models relevant to these higher levels of biological organization are added to the conventional set of toxicology models applied by pioneering ecotoxicologists.

Contaminant chemical form, phase association, and movement among components of the biosphere are also central issues in ecotoxicology because they determine exposure, bioavailability, and realized dose. The context of these biogeochemical studies has expanded in the last several decades to encompass issues of larger scale such as global movement of persistent organic pollutants (POPs) (Wania and Mackay, 1996).

From a practical vantage, ecotoxicology informs decision makers about ecological risks associated with contamination. Risk to ecological entities is estimated or predicted by combining exposure and effect information. Risk might involve diminished fitness of individuals, increased risk of local population extinction, a drop in species diversity, or reduced nutrient cycling or primary productivity. Because potential ecological end points are so diverse, the ecological risk framework tends to be more flexible than that of the conventional human health risk assessment (Fig. 30-2). This important role of ecotoxicology in ecological risk assessment (ERA) will be discussed in more detail below.

SOME DISTINCT ASPECTS OF EXPOSURE

Predicting exposure and effect is difficult for all relevant ecological entities. In contrast to human toxicology in which information about a few species might be used to predict harm to one (humans), ecotoxicology commonly uses sparse information for a few species to predict effects to many species and their interactions. Exposure pathways, bioavailability, bioaccumulation, and toxicant transfer for all relevant ecological entities are also difficult issues requiring considerable effort to adequately understand.

[1] Indirect effects are effects of toxicants mediated by another ecosystem component (Krivtsov, 2004) such as those that might occur to a flowering plant if a pesticide were to eliminate its primary insect pollinator.

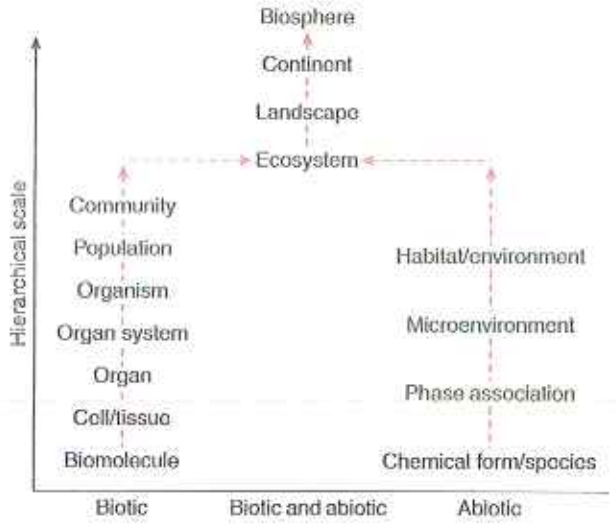

Figure 30-1. *Ecological scales relevant to ecotoxicology.* Solely biological scales relevant to ecotoxicology range from the molecular to the community levels; solely abiotic scales range from the chemical to the entire habitat. Biotic and abiotic components are usually combined at levels above the ecological community and habitat. The ecological community and physicochemical habitat combined to form the ecosystem. Ecological systems can be considered at the landscape scale, that is, the combination of marine, freshwater, and terrestrial systems at a river's mouth. Recently, the continental and biospheric scales have become relevant as in the cases of ozone depletion, acid precipitation, and global warming.

Relevant exposure routes are the conventional ingestion, inhalation, and dermal absorption. But unique features of exposure pathways must be accommodated for species that ingest a wide range of materials using distinct feeding mechanisms, breathe gaseous or liquid media using different structures, and come into dermal contact with a variety of gaseous, liquid, and solid media.

Prediction of oral exposure can be limited because species feed on different materials; however, conventional principles regarding oral bioavailability remain relevant. As an example, some birds are uniquely at high risk of lead poisoning because they ingest and then use lead shot as grit. Shot are ground together in their gizzards under acidic conditions, releasing significant amounts of dissolved lead (Kendall *et al.*, 1996). As true with humans (ie, the ionic hypothesis of Mathews, 1904), the dissolved form of lead is more available to do harm than solid lead shot. Similar high risk of lead poisoning is present for some raptors feeding on game birds whose tissues can contain lead shot (Wayland and Bollinger, 1999). Complex sorting of filtered materials on the gills of bivalve molluscs strongly influences the metal content and bioavailability of the material that eventually passes into their guts (Allison *et al.*, 1998). Some invertebrate species have elaborate feeding structures that are also involved in respiration (eg, lugworms and bivalve molluscs) or locomotion (copepods and other zooplankton species). Some zooplankton species feed and digest algal cells in such a way that only metals soluble in the algal cytosol are bioavailable (Reinfelder and Fisher, 1991). Unlike mammalian species, many invertebrate species are capable of sequestering large amounts of metals in intracellular granules (Mason and Nott, 1981). Incorporation of metals into granules by prey species reduces metal bioavailability to predators (Nott and Nicolaidou, 1993). Just as noted with 5-fluorouracil administration or chronic ethanol consumption, some pollutant exposures cause malabsorption by damaging the intestine wall. A relevant situation would be intestinal damage to otters caused by ingestion during grooming of oiled fur (Lipscomb *et al.*, 1996; Ormseth and Ben-David, 2000).

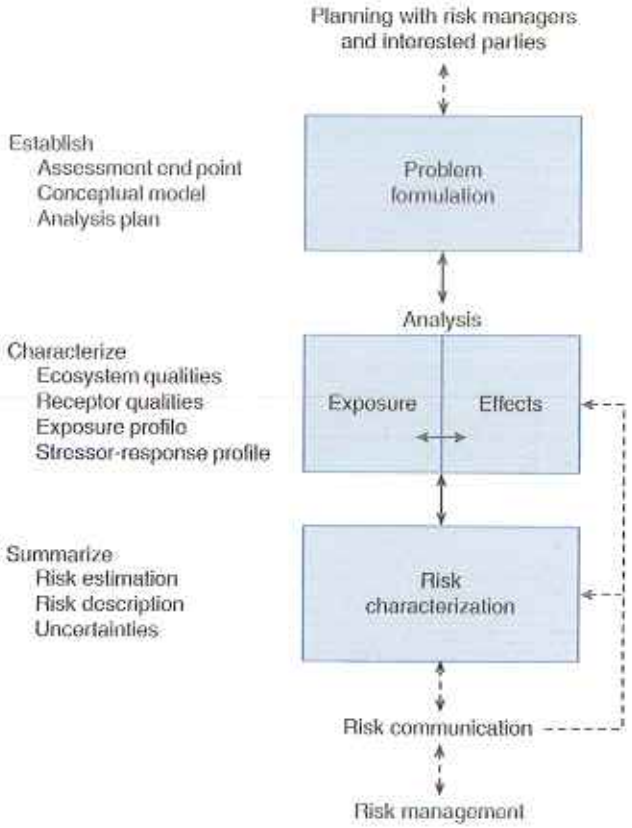

Figure 30-2. *The general form of an ecological risk assessment including problem formulation, analysis, and risk characterization stages.* Problem formulation is done in dialog with risk managers and stakeholders, and involves a clear statement of the ecological entity to be assessed, a conceptual model for the process, and a plan for conducting the assessment. The analysis stage involves exposure and effects characterizations. Using the context developed during problem formulation and information organized together in the analysis stage, a statement of risk and associated uncertainties are made in the risk characterization stage.

The principles remain the same in all of these cases but critical exposure pathway details are different.

Many techniques applied to determining human oral bioavailability are available to the ecotoxicologist; however, as just illustrated, modifications are needed for the many pathways for nonhuman species exposure. Typical are those associated with estimating contaminant bioavailability in aquatic environments. As an example, biomimetic and related extraction methods used to predict human oral bioavailability can be modified to predict sediment bound contaminant bioavailabilities. Sediments are placed into contact with biomimetic solutions (Chen and Mayer, 1998; Leslie *et al.*, 2002) or digestive fluids taken from organisms (Mayer *et al.*, 1996; Weston and Maruya, 2002), and the amount of extracted contaminant used to estimate bioavailability.

Estimation of chemical speciation is central to predicting bioavailability of water associated contaminants. Speciation can determine the bioavailability of dissolved metals. Movements of nonionic and ionizable organic compounds across the gut or gills are strongly influenced by lipid solubility and the pH-partition theory, respectively. Consequently, determination of a compound's lipophilicity or calculation of pH- and pK_a-dependent ionization facilitates some predictive capability for bioavailability. A common application of this approach would be estimation of water pH effects on ammonia toxicity as the consequence of the ease with which unionized ammonia passes through gills relative to

ionized ammonia (Lloyd and Herbert, 1960). The free ion activity model (FIAM) states that uptake and toxicity of cationic trace metals are best predicted from their free ion activity or concentration (Campbell and Tessier, 1996), although exceptions exist to this extension of the ionic hypothesis. Consequently, dissolved metal exposure assessments often begin by estimating the amount of a metal present as the free ion. Normally, such calculations require only thermodynamic modeling based on measured concentrations of dissolved cations and anions. Lipid partitioning is often used to predict dissolved, nonionizing organic compound accumulation in and effects to aquatic biota. The propensity for an organic compound to accumulate in aquatic organisms increases with lipid solubility as often described with a simple quantitative structure activity relationship (QSAR) (Neely *et al.*, 1974; Mackay, 1982; Chiou, 1985; Connell, 1990). The log of the octanol–water partition coefficient (log K_{ow}) is used to predict measures such as the bioconcentration factor (BCF; the quotient of the concentration in the organism and that in the water from which the organic compound is being accumulated):

AQ1

$$BCF = \frac{C_{Organism}}{C_{Water}}.$$

Bioavailability, bioaccumulation, or exposure concentrations for sediment-associated toxicants are also approached by considering chemical speciation and phase partitioning. Metals in sediments are either incorporated into one of many solid phases or dissolved in the interstitial waters surrounding the sediment particles. Bioavailabilities of metals in these different forms are difficult to predict (Luoma, 1989) but, nonetheless, various schemes have been applied to that end. Bioavailable metals have been estimated by normalizing sediment metal concentrations to easily extracted iron and manganese concentrations because solid iron and manganese oxides sequester metals in poorly bioavailable solid forms (Luoma and Bryan, 1978). Other chemical extraction methods have been applied with some success (Tessier *et al.*, 1984). A pragmatic method for predicting sediment metal bioavailability has emerged that is based on the assumption that the sediment metal form of most concern is the dissolved metal. Further, for many metals and sediments, the dissolved interstitial metal concentrations are determined by equilibrium between solid (iron and manganese) sulfides and the interstitial water:

$$Cd^{2+} + FeS_{Solid} \leftrightarrow CdS_{Solid} + Fe^{2+}.$$

Because the equilibrium so favors formation of metal sulfide (CdS in this case) at the expense of FeS, insignificant amounts of dissolved metal will be present in the interstitial waters if enough FeS is present. This premise has given rise to a standard technique for determining if sediments might contain enough metal to warrant concern (Di Toro *et al.*, 1990). First, a sediment aliquot is extracted with cold hydrochloric acid. Then the amounts of sulfide (acid volatile sulfides [AVS]) and simultaneously extracted metals (SEM) are measured in that extract. The difference between the SEM and AVS suggests whether or not enough metal will be dissolved in the interstitial waters to warrant concern. This method has enjoyed wide application and was recently refined by Di Toro *et al.* (2005) by including metal partitioning to sediment organic matter. Some ecotoxicologists such as Lee *et al.* (2000) suggest that further refinement remains to be done because metal exposure of organisms that ingest sediment particulates is not fully defined by interstitial water concentrations alone.

Bioavailability and accumulation of sediment-associated organic compounds are predicted with tools similar to those described for waters. The bioavailability of ionizable organic compounds can be approximated with the pH partition hypothesis that relates the availability of an ionizable compound to the diffusion of its unionized form through membranes as determined by pH and pK_a. Availability of nonionizing organic compounds for accumulation can often be estimated with its log K_{ow} and equilibrium partitioning theory as described already for accumulation from waters. The challenge with nonionizing organic compounds becomes adequately defining the phases between which the compound is partitioning. This might be done by estimating the partitioning of the compound between sediment solid phases and the interstitial water as done by Di Toro *et al.* (1991). Descriptions of nonionizing organic compound bioaccumulation from sediments can also entail normalization of concentrations to phases thought to be dictating partitioning:

$$\text{Biota sediment accumulation factor (BSAF)} = \frac{\mu g/kg \text{ lipid}}{\mu g/kg \text{ organic carbon}},$$

where the mass of compound in the organisms is divided by kilograms of organism-associated lipid and the mass of compound in the sediment is divided by kilograms of sediment-associated organic carbon.

Another issue of importance to the ecotoxicologist is the possibility of biomagnification, the increase in contaminant concentration as it moves through a food web. As will be described below, biomagnification can result in harmful exposures to species situated high in the food web such as birds of prey.

TOXICANT EFFECTS

While determining *exposure* comprises one half of the risk assessment paradigm, the other half, understanding chemical effects, lies at the heart of toxicology and, hence, comprises the focus of this chapter. The effects, or deleterious consequences of chemical exposures, can be enormously diverse as demonstrated by previous chapters, and investigated by numerous techniques. One approach to this complex topic of ecotoxicological effects, which we employ here, is to organize effects according to biological levels of organization. Thus, one may consider effects, in ascending order, at the subcellular (molecular and biochemical), cellular, organismal, population, community, and ecosystem levels of organization. As noted earlier, an important distinction between traditional biomedical, or human health-oriented, toxicology and ecotoxicology is the emphasis by the latter on higher levels of biological organization, specifically populations, communities, and ecosystems, while biomedical toxicology focuses on lower levels, from organismal and below. This difference arises from the focus of biomedical toxicology on one species and concerns for protecting the health of individuals of that species. Ecotoxicology, in contrast, deals with, theoretically at least, all species, and in line with other aspects of natural resource management, the primary concern is one of sustainability. That is, policies and regulations surrounding chemical effects in natural ecosystems are designed to protect ecological features such as population dynamics, community structures, and ecosystem functions. In this light, the individual organism is essentially viewed as expendable, as long as these higher level variables are protected. An exception here is that of endangered or threatened species, where the loss of an individual may have unacceptable legal or ecological consequences.

While higher levels of organization comprise the ultimate focus of those concerned with chemical pollution of natural systems, the science of ecotoxicology includes studies across the entire range. Studies at lower levels (cellular and below) provide

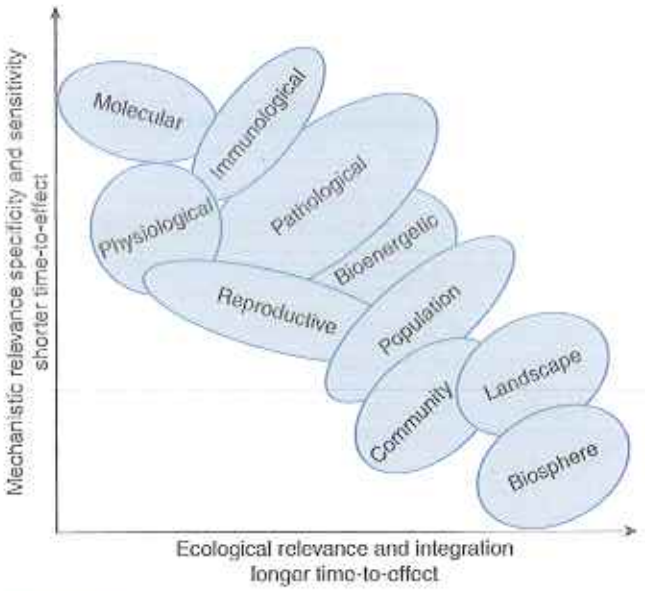

Figure 30-3. *Hierarchical types of effects considered in ecotoxicology, indicating relative attributes such as mechanistic versus ecological relevance and time lags between exposure and observable effect.* Based on artwork kindly provided by S. Marshall Adams, Oak Ridge National Laboratory.

insights into mechanisms of toxicity that can be valuable for making predictions among related compounds or species, and establishing cause–effect relationships in field studies, for generating useful "biomarkers" of chemical exposure and effect, and for providing insights into higher level, particularly population level, effects. Studies at the organism level have historically played a dominant role in regulatory ecotoxicology; many traditional bioassays, described later, can be viewed as organismal level queries. Again, understanding effects at the population and higher levels can be viewed as the "gold standard" in ecotoxicology. However, because these effects can require a relatively long time beyond the initial exposure of a system to emerge, their quantification is often difficult, and they generally do not serve to identify the nature of the stressor. Thus, the elucidation of effects of chemical pollutants, as well as other stressors, in natural systems draws on multiple approaches and conclusions are generally based on the weight of evidence available. Examples of effects, or end points, that can be measured spanning levels of organization and their relative sensitivities and ecological relevancies are illustrated in Fig. 30-3. The originator of this figure, Adams (Oak Ridge National Laboratory), has discussed the importance of integrating studies across levels of organization and mathematical approaches for accomplishing this (Adams, 2000). AQ2

In the following sections, we describe important chemical effects that have been addressed at different levels of biological organization in ecotoxicological contexts, including illustrative examples. It is beyond the scope of this chapter to provide discussions of all chemicals that have received ecotoxicological attention. Other chapters in this text, particularly those in Unit V, provide detailed information for most classes of chemicals of concern as pollutants of natural systems, albeit in a primarily mammalian context. Many of the effects described are relevant to other animals, and of course mammals do occur in natural systems! Some plant-specific effects will be addressed herein. It should be noted that, while we have employed a biological level of organization approach as a meaningful way to organize and convey a complex array of information, the phenomena we have categorized into various levels are ultimately interwoven, as will become apparent.

Molecular and Biochemical Effects

This lowest level of organization includes fundamental processes associated with the regulation of gene transcription and translation, biotransformation of xenobiotics, and the deleterious biochemical effects of xenobiotics on cellular constituents including proteins, lipids, and DNA. These effects have been described elsewhere throughout this book in various contexts related to human health. Here we will highlight some aspects of subcellular effects that have received particular attention in the context of ecotoxicology. Research in this area has been performed for a variety of reasons, including the elucidation of mechanisms of adaptation and toxicity, understanding species similarities and differences (eg, to compare selected wildlife species with standard mammalian models, and to identify particularly sensitive species), and to develop useful biomarkers of chemical exposure and toxicity for environmental assessments. More in-depth discussions are provided in monographs such as Hoffman *et al.* (2003), Newman and Unger (2003), Mommsen and Moon (2005), and Di Giulio and Hinton (in press).

Gene Expression and Ecotoxicogenomics

A long-standing mechanistic issue in toxicology concerns chemical effects on gene and protein expression. Xenobiotics can affect gene transcription through interactions with transcription factors and/or the promoter regions of genes that bind transcription factors in the process of activating transcription. In the context of environmental toxicology, perhaps the most studied xenobiotic effects involve ligand-activated transcription factors. These intracellular receptor proteins recognize and bind specific compounds, thus forming a complex that binds to specific promoter regions of genes, thereby activating transcription of mRNAs, and ultimately translation of the associated protein. Two examples of substantial importance in ecotoxicology that illustrate these interactions involve the estrogen receptor (ER) and the aryl hydrocarbon receptor (AHR).

Estrogen Receptor A number of chemicals have been shown to perturb various components of the endocrine system, and the identification and elucidation of "endocrine disruptors" has been a subject of much research and regulatory action in recent years, in the contexts of both human and wildlife health (see Chap. 10; Rotchell and Ostrander, 2003). Perhaps the most studied component of the vertebrate endocrine system in this context is the ER, particularly ER-α, and responses associated with it. The dominant natural ligand for this nuclear receptor is estradiol (E2); binding of E2 with ER produces a complex that can then bind to estrogen response elements (ERE) of specific genes that contain one or more EREs, thereby causing gene transcription (see Fig. 30-4). Genes regulated in this manner by E2–ER play various important roles in, for example, sexual organ development, behavior, fertility, and bone integrity (Deroo and Korach, 2006).

A number of chemicals including certain drugs and environmental pollutants can serve as ligands for ER; in most cases these "xenoestrogens" activate gene transcription, that is, similar to E2, acting as receptor agonists. The first xenoestrogen identified was diethylstilbestrol (DES), a drug used to prevent miscarriage in the 1940s to the 1970s until it was discovered to have profound developmental effects in some offspring of women receiving the treatment (Trimble, 2001). In recent years, a number of environmental pollutants with estrogenic activity have been identified, including certain chlorinated hydrocarbon insecticides (eg, DDT, methoxychlor, endosulfan), surfactants (nonylphenol), some

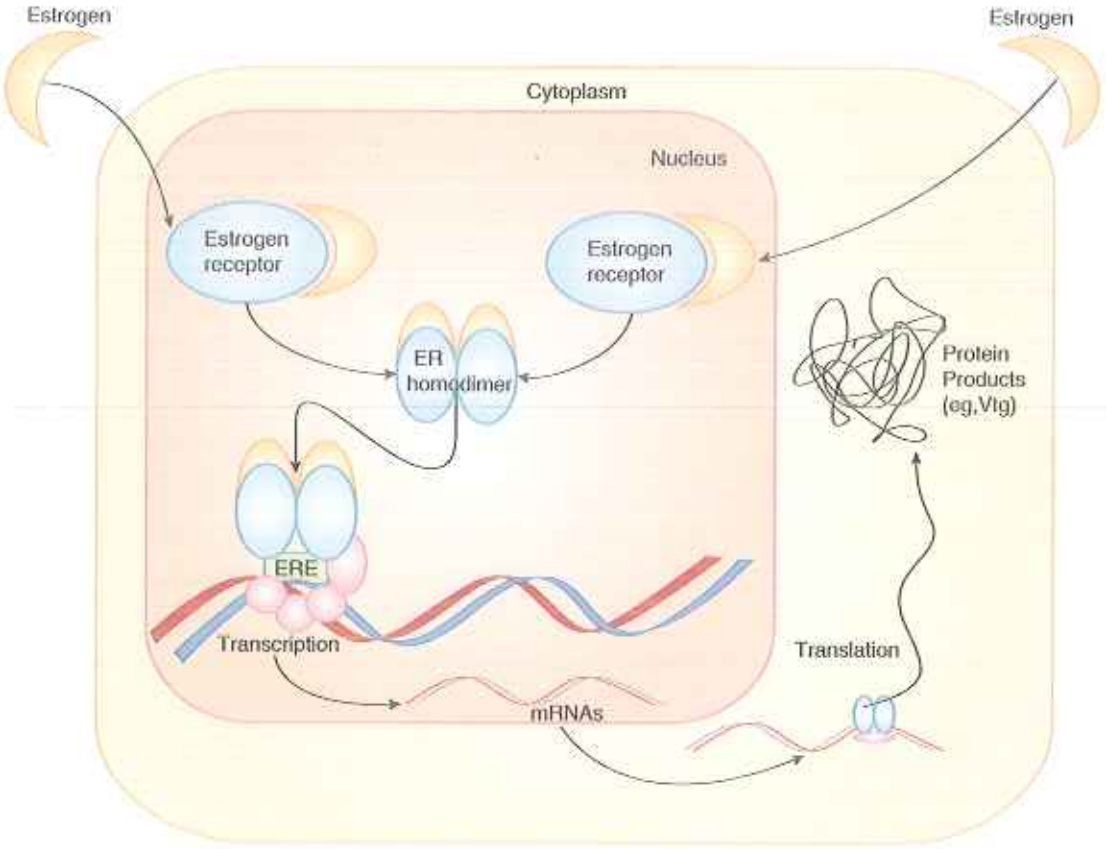

Figure 30-4. *A simplified model of estrogen receptor signaling including the estrogen receptor (ER), which on binding to native hormone ligand (estrogen) or xenoestrogen (see Fig. 30-5) forms the transcriptionally active ER homodimer complex that binds to genes containing estrogen response elements (ERE) and thereby upregulates transcription and translation of genes/proteins regulated by the system, such as vitellogenin (Vtg).* Kindly provided by Carla Rosenfeld, Duke University.

polychlorinated biphenyls (PCBs), bisphenol A (used in plastic manufacturing), and ethinyl estradiol, a synthetic estrogen used in birth control pills and observed in municipal effluents and surface waters (Shelby *et al.*, 1996; Larsson *et al.*, 1999; van der Oost *et al.*, 2003; see Fig. 30-5). With the exception of ethinyl estradiol, these pollutants exhibit relatively low binding affinities to ER, as compared with E2 or DES (Shelby *et al.*, 1996); however, environmental exposures may be sufficient to perturb reproduction or development.

Evidence for such "endocrine disruptions" by environmental xenoestrogens appears to be overall stronger for wildlife than for humans, likely due to instances of elevated exposures that are less prone to confounding factors than is typically the case for human exposures. Also, egg-laying vertebrates provide a unique biomarker of estrogen exposure that has contributed to ecotoxicological studies in this area. Vitellogenin (Vtg) is a protein that is normally produced by the liver of females and transported via the bloodstream to the ovary where, as a key component of yolk, it provides nourishment to the developing embryo. The production of Vtg is regulated by the estrogen–ER system. Interestingly, males of egg-laying vertebrate species contain the molecular machinery to produce Vtg, but production and circulating levels are normally very low, due to low titers of estrogen. However, exposures of males to estrogen and xenoestrogens upregulate Vtg production, which can be readily measured in blood samples. Consequently, elevated Vtg in males of these species is a useful biomarker of estrogenic chemical exposures (Sumpter and Jobling, 1995; see the section "Biomarkers"). Examples include rainbow trout (*Oncorhynchus mykiss*) caged in surface waters below industrial or municipal effluent sources enriched in alkylphenolic surfactants in the United Kingdom (Harries *et al.*, 1997) or natural and synthetic estrogens in Sweden (Larsson *et al.*, 1999), and whitefish (*Coregonus lavaretus*) caged near paper mill effluents in Finland (Mellanen *et al.*, 1999). While the bulk of research related to estrogenic compounds in natural systems has focused on fish, this approach has merit for other egg-laying vertebrates (Lorenzen *et al.*, 2003; Huang *et al.*, 2005), and for some invertebrates that produce Vtg-like proteins (Porte *et al.*, 2006).

Aryl Hydrocarbon Receptor The AHR is a member of the basic helix–loop–helix Per ARNT Sim (bHLH-PAS) family of receptors/transcription factors that play roles in development, as sensors of the internal and external environment in order to maintain homeostasis, and in establishment and maintenance of circadian clocks (Denison and Nagy, 2003; Hahn *et al.*, 2005). The AHR is among the most intensively studied receptors in toxicology due to its role in regulating a number of genes coding for proteins involved in xenobiotic metabolism, and its responsiveness to a number of widespread environmental contaminants, as well as some drugs and endogenous compounds. The AHR is a ligand-activated cytosolic receptor that on binding to a ligand traverses to the nucleus where it complexes with another transcription factor, the AHR nuclear translocator (ARNT) protein (Fig. 30-6) to form a transcriptionally active dimer. This AHR–ARNT complex binds to promoter sequences of genes regulated by the AHR system; these promoters are most often referred to as "xenobiotic response elements" (XRE) or "dioxin response elements" (DRE).

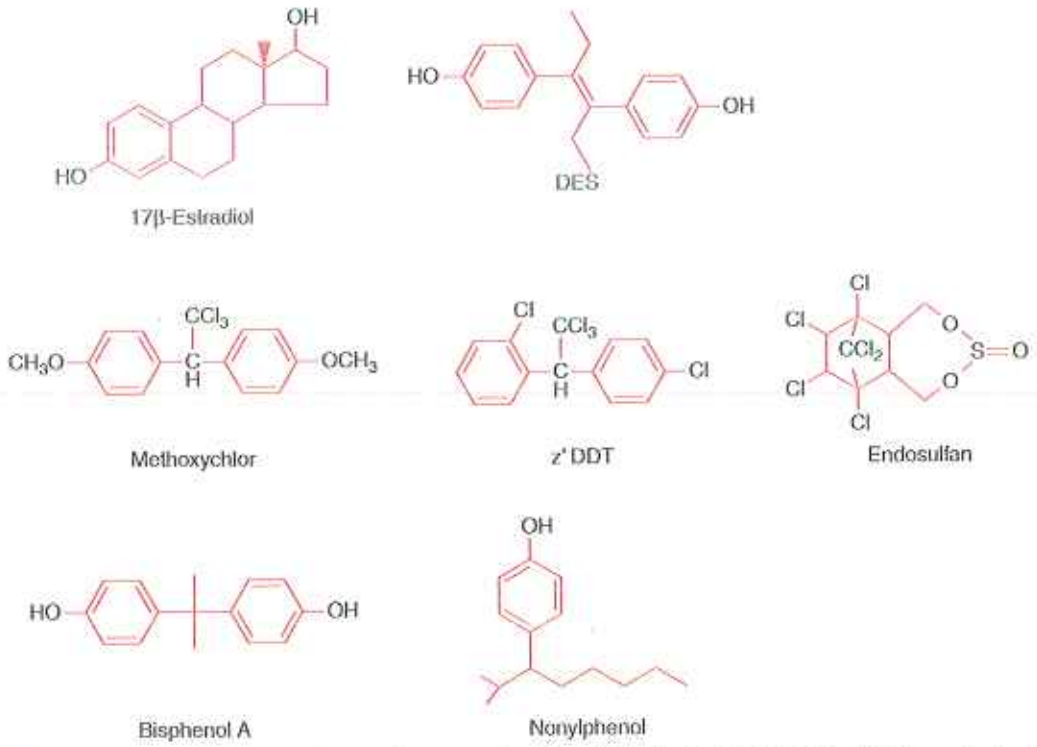

Figure 30-5. *Estrogen receptor agonists, including a native estrogen (17β-estradiol), the drug diethylstilbestrol (DES), a surfactant component (nonylphenol), an industrial intermediate (bisphenol A), and several pesticides (remainder).*

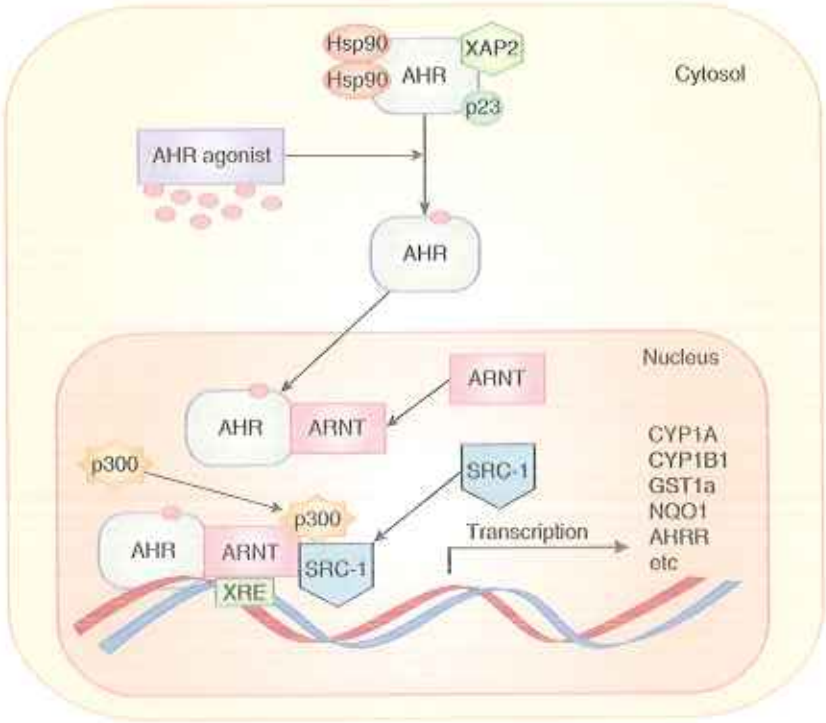

Figure 30-6. *A simplified model of the aryl hydrocarbon receptor (AHR) including activation by AHR ligands (see Fig. 30-7) that allows for dimerization with the AHR nuclear transporter (ARNT) that forms the transcriptionally active complex that binds to xenobiotic response elements (XRE) and thereby upregulates a number of genes, including several involved in biotransformation, indicated here, as well as the AHR repressor (AHRR) that provides negative feedback control of the system.* Hsp90, XAP2, and p23 are chaperone proteins; SRC-1 and p300 are examples of coregulator proteins involved in transcription. Kindly provided by Carrie Fleming and Carla Rosenfeld, Duke University.

Characterized genes that are upregulated by the AHR system in large part code for enzymes involved in the metabolism of lipophilic chemicals, including organic xenobiotics and some endogenous substrates such as steroid hormones. These enzymes include specific cytochrome P450s (mammalian CYP1A1, 1A2, and 1B1 and their counterparts in other vertebrates), a glutathione transferase (GST), a glucuronosyl transferase (UDPGT), an alcohol dehydrogenase (ALD), and a quinone oxidoreductase (NQO). In addition to these enzymes, AHR–ARNT upregulates the AHR repressor protein (AHRR), which then competes with AHR for ARNT. This results in a transcriptionally inactive complex and effectively provides a negative feedback loop for controlling AHR-mediated gene transcription (Hahn *et al.*, 2005). The biotransformation enzymes regulated by the AHR system are described in detail in Chap. 6. Briefly, they play key roles for transforming many lipophilic chemicals, including numerous common organic contaminants, into more water-soluble, and hence excretable, products. However, this biotransformation activity can result in production of highly reactive products that are more toxic than their parent compounds, that is, "activation."

Of particular relevance to the present discussion, however, is the ability of some ubiquitous pollutants to act as AHR ligands and markedly upregulate gene transcription via the AHR–ARNT signaling pathway described above (Denison and Nagy, 2003). In some cases, this can be interpreted as an adaptive response—the organism is reacting to exposure to a lipophilic xenobiotic in order to enhance its elimination. However, as noted above, biotransformation can also lead to enhanced toxicity of some substrates. The two major classes of pollutants that have members that act as ligands for the AHR and upregulate gene expression (and thereby "induce" biotransformation enzymes) and have received the greatest attention in ecotoxicology are the polycyclic aromatic hydrocarbons (PAHs) and the polyhalogenated aromatic hydrocarbons (pHAHs); examples of both are provided in Fig. 30-7. The most studied pHAHs are particular "coplanar" PCBs and chlorinated dioxins. Whereas some PAHs and pHAHs share the ability to activate the AHR, important differences between these classes exist. PAHs, whether they are ligands for the AHR or not, are overall very good substrates for the biotransformation systems upregulated via the AHR, which can act to both detoxify and enhance the toxicity of some PAHs (discussed in the section "Cancer"). In general, pHAH type AHR ligands are more potent AHR ligands and enzyme inducers than PAHs, but due to extensive halogenation are much poorer substrates for biotransformation. One of the most potent ligands for the AHR is the dioxin, 2,3,7,8-tetrachlorodibenzodioxin (TCDD), which is highly recalcitrant to biotransformation (Denison and Nagy, 2003).

In similarity with xenoestrogen mediated inductions of Vtg via the ER, the inducibility of biotransformation enzymes via the AHR by xenobiotics has been used for biomonitoring. In this regard, enzymatic activities associated with the CYP1As have been the most widely used AHR-related biomarker, particularly an activity that appears highly specific for these CYPs, ethoxyresorufin *O*-deethylase (EROD), which is most often measured in liver tissue of vertebrates. Elevated activities of EROD in various vertebrates have been associated with exposures to PCBs, dioxins, PAHs, and complex mixtures of these associated with, for example, harbor sediments, municipal effluents, paper mill effluents, refinery effluents, and crude oil spills (Custer *et al.*, 2001; van der Oost, 2003; Miller *et al.*, 2005). Invertebrate AHR homologues examined do not bind to ligands similarly to vertebrate AHRs and do not demonstrate protein inductions analogous to those observed in vertebrates (Butler *et al.*, 2001; Hahn, 2002; Chaty *et al.*, 2004). PAH and pHAH toxicities and the potential roles played by their interactions with the AHR will be discussed at various points in subsequent sections of this chapter, and related discussions from a human health standpoint appear in other chapters.

Genomics and Ecotoxicogenomics Recent advances in gene sequencing and associated techniques for investigating mechanism underlying gene expression have revolutionized molecular biology. These advances are rapidly permeating many areas of biological research, including toxicology and environmental science. Underlying these advances are very large projects to sequence the entire genomes of various species, such as the highly publicized Human Genome Project that was completed in 2003 (Little, 2005). Other species that have been completely or largely sequenced include the mouse, rat, cow, dog, chimpanzee, chicken, zebrafish (*Danio rerio*), puffer fish (*Fugu rubripes*), medaka (*Oryzias latipes*), fruit fly (*Drosophila melanogaster*), a sea urchin (*Strongylocentrotus purpuratus*), a soil nematode (*Caenorhabditis elegans*), a yeast (*Saccharomyces cerevisiae*), and rice (*Oryza sativa*), and the number of species sequenced is anticipated to expand rapidly (see www.genome.gov; Crollius and Weissenbach, 2005). Genome sequencing set the stage for genome-wide analysis of gene expression ("transcriptomics");

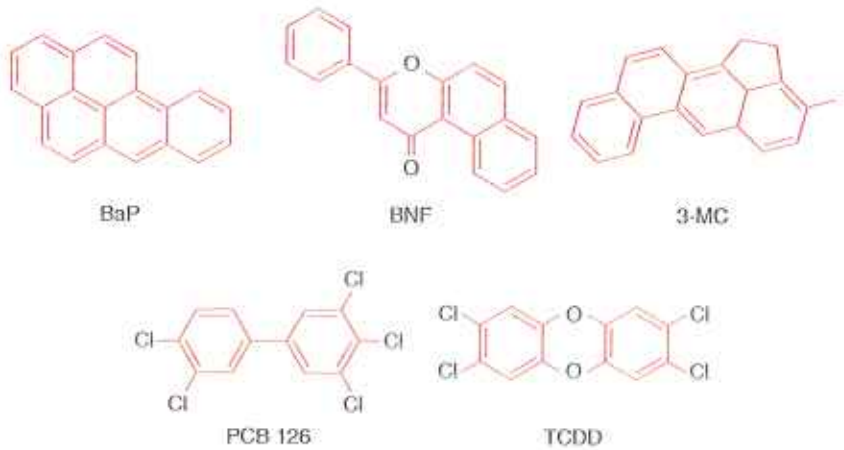

Figure 30-7. *Representative ligands of the aryl hydrocarbon receptor, including 2,3,7,8-tetrachlorodibenzodioxin (TCDD), a coplanar polychlorinated biphenyl (PCB 126), two polycyclic aromatic hydrocarbons (benzo(a)pyrene [BaP] and 3-methylcholanthrene [3-MC]), and a flavone (β-naphthoflavone [BNF]).*

cDNAs for known genes can be spotted on glass slides, or chips, resulting in "microarrays" that can be employed to quantify relative levels, that is, expression, of mRNAs for those genes in samples of interest. The study of changes in gene expression arising from chemical exposures is a key component of "toxicogenomics" (Schmidt, 2002). Microarrays for genomes or genome components of interest (such as genes associated with stress responses, carcinogenesis, and development) are commercially available for many species, and rapidly expanding. Also advancing rapidly are related analyses of global changes in proteins (the translation products of mRNAs), referred to as "proteomics," and resulting metabolite profiles (amounts of sugars, lipids, amino acids, etc, in various tissues that are controlled in part by enzyme activities), referred to as "metabolomics." A major complexity in these global analyses is the extremely large data sets that arise, for example, when one examines the responses of thousands of genes from organisms exposed to one or more concentrations of a chemical at one or more time points. This has led to the development of the field of "bioinformatics" that includes the application of sophisticated statistical and computing approaches for revealing biologically meaningful patterns of gene expression such as relationships to cellular signaling pathways. "Omics" is a term used to refer collectively to these interrelated approaches (ie, transcriptomics, proteomics, metabolomics, and bioinformatics).

Omics have spread into the science and applications of ecotoxicology, collectively termed ecotoxicogenomics. As is the case for human health-oriented toxicogenomics, ecotoxicogenomics has great potential for elucidating impacts of chemicals of ecological concern and ultimately for playing an important role in ERAs and regulatory ecotoxicology (Snape *et al.*, 2004; Ankley *et al.*, 2006; Watanabe and Iguchi, 2006). Specific areas to which this emerging field can contribute include prioritization of chemicals investigated in ERAs, identification of modes of action of pollutants, identification of particularly sensitive species, and effect prediction at higher levels of organization. As in other areas of ecotoxicology, a major complexity faced is the vast array of species of potential concern. This is a particularly problematic issue in ecotoxicogenomics that requires substantial species specific molecular information. However, as mentioned earlier, the number of ecologically relevant species for which this information is becoming available is expanding rapidly, and is likely to accelerate as tools are refined. Moreover, as information grows, genomic approaches hold great promise for identifying appropriate surrogate species for laboratory studies used in basic ecotoxicological research and in support of regulatory ecotoxicology (Benson and Di Giulio, 2006).

Protein Damage The study of chemical effects on proteins, particularly enzymes, has a long history in toxicology. Of particular interest within ecotoxicology are the inhibitions of acetylcholinesterase (AChE) by certain pesticides and of delta-aminolevulinic acid dehydratase (ALAD) by lead. AChE degrades the neurotransmitter acetylcholine, and in so doing, controls nerve transmission in cholinergic nerve tracts. The widely used organophosphate and carbamate classes of insecticides kill by inhibiting AChE, and this mechanism is operative for "nontarget" organisms including invertebrates, wildlife, and humans. (See Chap. 22 for a detailed discussion of these pesticides and AChE inhibition.) Of particular ecological concern have been the ingestions of AChE-inhibiting insecticides with food items or granular formulations (mistaken as seed or grit) by birds and exposures to aquatic animals from agricultural runoff (Mineau, 1991; Carr *et al.*, 1997; Wilson *et al.*, 2001). In many field studies, effects of these insecticides on mortality, and relationships between AChE inhibition and mortality, have been of primary concern. However, relationships between AChE inhibition and important sublethal impacts such as behavior have also been observed (Sandahl *et al.*, 2005). ALAD catalyzes the rate-limiting step of heme synthesis, a key component of cytochromes, hemoglobin, and myoglobin, and ALAD activity is very sensitive to inhibition by lead (ATSDR, 1999). This sensitivity has been exploited widely as a biomarker for lead exposure in humans and wildlife. In wildlife, concerns for lead exposure have included ingestion by birds of spent lead shot used in hunting (Kendall *et al.*, 1996), accumulation of lead by wildlife living near highways (Birdsall *et al.*, 1986), and aquatic organisms inhabiting surface waters contaminated by lead from mine runoff and other industrial activities (Schmitt *et al.*, 2005). In addition to enzyme inhibition, chemicals can damage proteins in other ways, including oxidative damage as described below, and by forming stable adducts similar to those formed with DNA, also discussed below. AQ1

Oxidative Stress The classic depiction of aerobic respiration shows molecular oxygen (O_2) as the terminal electron acceptor, with its reduction resulting ultimately in water as high energy intermediates (NADH, $FADH_2$) are oxidized and cellular energy is captured as ATP. This reduction of O_2 to H_2O requires four electrons that are sequentially added; this process is tightly coupled so that the one-, two-, and three-electron intermediates are released at low amounts (less than 0.1% of O_2 inspired; Fridovich, 2004). These intermediates are, in sequence, the superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$). This tight coupling is fortunate because these intermediates, produced during aerobic respiration and other O_2 consuming or producing processes (such as photosynthesis and CYP-mediated biotransformations), are potentially deleterious products that can indiscriminately damage cellular components; hence, they are referred to as "reactive oxygen species" (ROS). Some ROS, including $O_2^{\bullet-}$ and $^{\bullet}OH$, are free radicals, that is, they possess an unpaired electron; among ROS, $^{\bullet}OH$ is particularly reactive and toxic. In order to defend themselves from the damaging effects of ROS, all aerobic organisms have evolved complex antioxidant defense systems that include enzymatic and nonenzymatic components (Halliwell and Gutteridge, 1999). Antioxidant enzymes include superoxide dismutases that convert $O_2^{\bullet-}$ to H_2O_2, catalases and peroxidases that detoxify peroxides including H_2O_2, and enzymes involved in the production and maintenance of reduced glutathione (GSH) such as glutamate–cysteine ligase (GCL) and glutathione reductase (GR). Low-molecular-weight, nonenzymatic antioxidants include vitamins A, C, and E, and GSH.

Oxidative stress has been defined as "a disturbance in the prooxidant–antioxidant balance in favor of the former, leading to potential damage" (Sies and Cadenas, 1985)—that is, the point at which the production of ROS exceeds the capacity of antioxidants to prevent damage. Numerous environmental contaminants can act as prooxidants and enhance the production of ROS. The resulting oxidative damage can account wholly or partially for toxicity (Halliwell and Gutteridge, 1999). Mechanisms by which chemicals can enhance ROS production include redox cycling, interactions with electron transport chains (notably in mitochondria, microsomes, or chloroplasts), and photosensitization.

Redox cycling is perhaps the most common mechanism by which a diverse array of chemicals including many environmental pollutants generates intracellular ROS. Redox cycling chemicals include diphenols and quinones, nitroaromatics and azo compounds, aromatic hydroxylamines, bipyridyliums, and certain metal chelates, particularly of copper and iron (Di Giulio *et al.*, 1989; Halliwell and Gutteridge, 1999). These include compounds

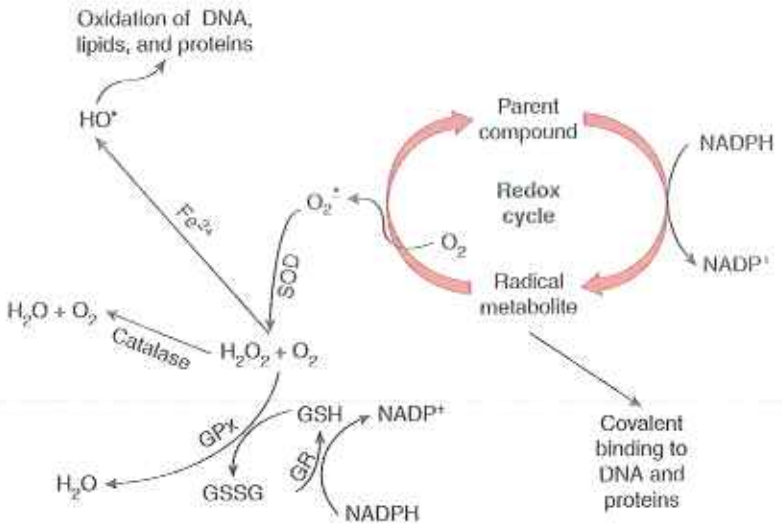

Figure 30-8. *Overview of oxidative stress, including reactive oxygen species stimulation initially by redox cycling, key antioxidant defenses, and potential deleterious biochemical effects.* "Parent compound" in redox cycle could include a number of chemicals such as quinones, nitroaromatics, azo dyes, paraquat and diquat, and transition metal chelates.

of broad industrial use, many pesticides, ubiquitous elements, and metabolic products of numerous pollutants. In the redox cycle, the parent compound accepts an electron from a reduced cofactor, such as NADH or NADPH; this reaction is typically catalyzed by a reductase such as xanthine oxidase or cytochrome P450 reductase (Kappus, 1986). In the presence of O_2, the unpaired electron of the radical metabolite is donated to O_2, yielding $O_2^{\bullet}$ and regenerating the parent compound; importantly, the parent compound can repeat this cycle until it is cleared or metabolized to an inactive product. In the course of each redox cycle, two potentially deleterious events occur—a high-energy reducing equivalent is expended (the oxidation of NADPH to $NADP^+$, eg) and an oxygen radical is produced. A generalized redox cycle that includes associations with cellular toxicities and antioxidant defenses comprises Fig. 30-8.

Additionally, pHAH AHR ligands such as coplanar PCBs and TCDD can enhance ROS production, possibly by inducing CYP1A activity and concomitantly interfering with electron flow mediated by these enzymes (Schlezinger and Stegeman, 2001). PAHs can also upregulate CYP1A and can enhance the production of ROS-generating redox-active quinone metabolites, in contrast to uncoupling mechanisms proposed for pHAHs; Nebert *et al.* (2000) discuss potential mechanisms by which AHR agonists can produce oxidative stress. The herbicide paraquat is phytotoxic due to interference with chloroplast electron transport. Interestingly, it is a very potent lung toxicant because of its specific uptake by this tissue and subsequent redox cycling (Halliwell and Gutteridge, 1999). Another important mechanism particularly significant in aquatic systems is photosensitization. Ultraviolet (UV) radiation (specifically UVB and UVA) can penetrate surface waters to depths dependent on the wavelength of the radiation and the clarity of the water. The UV radiation generates ROS and other free radicals via excitation of photosensitizing chemicals, including common pollutants of aquatic systems (Larson and Weber, 1994). For example, due to photosensitization, many PAHs are orders of magnitude more acutely toxic to aquatic organisms in the presence of UV radiation than in its absence (Arfsten *et al.*, 1996; Ankley *et al.*, 1997). The ecological relevance of photosensitization, however, is controversial (McDonald and Chapman, 2002).

As noted, ROS are generally indiscriminant molecules and can potentially damage any cellular component. Well-characterized biochemical impacts of ROS include oxidations of unsaturated lipid components of membranes ("lipid peroxidation"), oxidations of amino acids and proteins (resulting in, eg, the addition of carbonyl groups), and DNA oxidations resulting in products such as 8-hydroxy-guanosine and thyme glycol (Halliwell and Gutteridge, 1999). Another important impact is perturbed redox status (Schafer and Buettner, 2001). Healthy cells typically maintain high ratios of cofactors in their reduced, high energy state relative to their oxidized state (eg, NADH/NAD^+, NADPH/$NADP^+$, and GSH/GSSG), as the reduced forms are those most employed for energy production, biosynthesis, and antioxidant defense, for example. The ROS can drive redox status to a more oxidized state by several direct and indirect mechanisms, potentially reducing cell viability. These ROS-mediated impacts and others have been associated with a number of human diseases including atherosclerosis, arthritis, cancer, and neurodegenerative diseases such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis (Halliwell and Gutteridge, 1999). With the exception of cancer (see below), the role of ROS in specific diseases in wildlife has received little attention. However, numerous studies have documented oxidative stress-mediated biochemical and cellular effects in wildlife associated with environmental contamination (Bainy *et al.*, 1996; Livingstone, 2001; van der Oost, 2003; Dorval *et al.*, 2005). As with humans and various animal models for human disease, it is reasonable to assume that oxidative stress comprises an important mechanism accounting in part for the toxicity of diverse pollutants to free-living organisms. Also, oxidative stress is involved in the effects of air pollutants on plants and likely plays a role in forest diebacks observed downwind of industrialized areas (Richardson *et al.*, 1989; Hippeli and Elstner, 1996).

DNA Damage The importance of DNA as a molecular target in toxicology is indicated by the devotion of Chap. 9 of this text to genetic toxicology. As indicated, perhaps the most pressing human health issue associated with xenobiotic–DNA interactions is cancer. Cancer is also an important health outcome associated with chemical exposures in wildlife, particularly for bottom-dwelling fishes, as discussed in the section "Cancer." Of greater concern in ecotoxicology versus human health are other, multigenerational effects of pollutants on genetic structures and resulting phenotypes of

populations and communities, through both direct effects on DNA (mutations) and indirect effects (selection); this topic is discussed in the subsection "Population" in the section "Toxicant Effects."

Chemical contaminants can damage DNA through several mechanisms, including the formation of DNA–xenobiotic adducts, by causing strand breaks, and by oxidations of DNA bases. In the context of ecotoxicology, the most widely studied form of damage has been the formation of stable DNA adducts, particularly by PAHs. In order to form these adducts, PAHs must first be activated to reactive metabolites by enzyme systems such as the cytochrome P450s. The bulk of PAHs metabolized to various oxidized products (such as phenols, diols, and epoxides) is subsequently conjugated by phase II enzymes (such as GSH, sulfate, and glucuronosyl transferases; see Chap. 6); however, some fraction can react with DNA, mainly through covalent bonding with DNA bases. The most studied example of this is benzo(*a*)pyrene (BaP), which can be metabolized to the highly reactive benzo(*a*)pyrene diol epoxide (BPDE) that can bond to DNA. In field studies, the resulting large adducts of DNA with BPDE and other activated PAHs and related compounds can be measured with the highly sensitive ^{32}P-postlabeling assay (Phillips, 1997). This technique has been used extensively to monitor DNA adducts in benthic fish and bivalves inhabiting systems contaminated with hydrocarbons, particularly PAHs (Maccubbin, 1994; Reichert *et al.*, 1998; Shugart, 2000; Amat *et al.*, 2004). Other forms of DNA damage that have been investigated in ecotoxicological studies include DNA strand breaks and oxidized DNA bases (Shugart, 2000; Malins *et al.*, 2006).

Once DNA damage has occurred, whether from chemical exposures or other causes (respiration, UV radiation, viral interactions, normal wear and tear), several subsequent outcomes can occur, including the following: the damage can be properly repaired, the damage can lead to cell death, or a resulting change in DNA structure (base sequence) can become fixed and passed on to daughter cells, that is, mutation occurs. Complex DNA repair systems have been elucidated in prokaryotic and eukaryotic organisms (see Chaps. 8 and 9), and while these systems have received relatively little attention in species of ecological relevance, it is a safe assumption that these conserved systems are qualitatively similar across diverse phyla. Overall, these systems exhibit a remarkable capacity for surveying the cellular genome, detecting damage such as oxidations, adducts, and strand breaks, and repairing the damage by, for example, removing a damaged base and replacing it with the correct base. However, misrepair does sometime occur, with the result that an incorrect base is incorporated. Depending on the gene involved and the site within the gene, this change may lead to cell death, or may result in a mutation that may have no effect (occurs at noncritical base sequence) or one that leads to functional change in the protein coded by the gene. Some chemicals cause cancer by mutating genes that play pivotal roles in cellular growth and differentiation, particularly oncogenes and tumor suppressor genes. Examples of discoveries of activated genes (in liver tumors) in field studies include the *K-ras* oncogene in tomcod (*Microgadus tomcod*) from the Hudson River, New York (Wirgin *et al.*, 1989), and in winter flounder (*Pseudopleuronectes americanus*) collected from Boston Harbor (McMahon *et al.*, 1990), and the retinoblastoma (*Rb*) tumor suppressor gene in the marine flatfish, dab (*Limanda limanda*), from the United Kingdom (du Corbier *et al.*, 2005).

Cellular, Tissue, and Organ Effects

Cells Cellular organelles that have received attention as targets in species of ecological interest include mitochondria, lysosomes, and nuclei. Most free-living organisms routinely experience energy deficits. For example, food resources are often highly depleted during the winter for many animals, which adapt by conserving energy (by hibernating or lowering metabolism) or by storing energy beforehand (as the case for many migratory birds). Thus, effects of pollutants on mitochondrial energy metabolism can be of particular importance to wildlife. For example, Sokolova and coworkers (Sokolova, 2004; Cherkasov *et al.*, 2006) have elegantly described the effects of cadmium on several aspects of mitochondrial function in isolated gill and hepatopancreas cells from the eastern oyster (*Crassostrea virginica*), and noted a marked synergy between the metal and increasing environmental temperatures. Lysosomes are involved in the degradation of damaged organelles and proteins, and also sequester a wide variety of environmental contaminants, including metals, PAHs, and nanoparticles (Moore *et al.*, 2005). The accumulation of xenobiotics by lysosomes can elicit membrane damage, or "membrane instability," which has been used as an early warning measure of pathological chemical effects in both invertebrates and vertebrates (Hwang *et al.*, 2002; Kohler *et al.*, 2002; Moore *et al.*, 2006).

In addition to specific damage to DNA bases described above, chemical effects on nuclei have been examined in ecological contexts with additional techniques. Micronuclei are chromosomal fragments that are not incorporated into the nucleus at cell division, and chemical exposures can markedly increase their frequency. Elevated micronuclei numbers have been observed, for example, in fish erythrocytes from polluted coastal sites in California (Hose *et al.*, 1987) and in hemocytes in clams from a PCB polluted harbor in Massachusetts (Dopp *et al.*, 1996). Also, a standardized higher plant (*Tradescantia*) assay for micronuclei has been used for monitoring air pollution (Solenska *et al.*, 2006). A cell-based assay that has been used widely in environmental applications is the comet assay. In this assay, cells are imbedded in agarose, lysed and subjected to gel electrophoresis, and the features of the resulting "comet's tail" on the gel used to assess DNA damage. With appropriate manipulations, the comet assay can be employed to detect and distinguish among a variety of genotoxicities including strand breaks, oxidative damage, and adducts (Moller, 2006). It has been used in a variety of field applications, particularly with bivalves (Steinert, 1999; Nigro *et al.*, 2006), fish (Heuser *et al.*, 2004), and mice (Hushy and McBee, 1999). AQ2

Histopathology The detailed microscopic analysis of the structure of cells and tissues can provide important links among chemical exposures, cellular targets and mechanisms, and effects at the organismal level (Hinton, 1994). Moreover, the determination that tissue damage has occurred as demonstrated by histopathological analysis is extremely useful for inferring that a significant deleterious effect has occurred. However, the substantial expertise required for proper histopathological analyses of this nature and the oftentimes time- and labor-intensive nature of these analyses has perhaps limited the application of this powerful approach in ecotoxicological contexts. Nevertheless, histopathological analysis has played an important role in confirming chemically mediated tissue damage in numerous laboratory and field studies. For example, Pacheco and Santos (2002) integrated histopathological analysis with biochemical studies of the effects of various environmental contaminants on the European eel (*Anguilla anguilla*), and Devlin (2006) similarly incorporated this approach in studies of the effects of methylmercury in fathead minnows (*Pimephales promelas*). Handy *et al.* (2002) relied on histopathology in a study of fish health in rivers in southern England. Wester *et al.* (2002) reviewed the application and potential contributions of histopathology in aquatic toxicology, particularly in the context of small fish models. In subsequent

sections concerning organismal-level impacts, other examples of the use of histopathology will be provided.

Target Organs Descriptions of chemical impacts on all organ systems of the myriad species relevant to ecotoxicology are beyond the scope of this chapter. Other chapters in this volume address key target organs in the mammalian context, and much of this is relevant to other vertebrates. Target organ toxicology is also the subject of comprehensive reviews by Schlenk and Benson (2001) concerning marine and freshwater fishes, and by Gardner and Oberdorster (2005) concerning reptiles. Relevant information in marine mammals was reviewed in Vos *et al.* (2002). We are unaware of similar reviews for birds or invertebrates. The unique properties of the avian respiratory system and its utility for investigating respiratory system toxicity and air pollution were reviewed by Brown *et al.* (1997).

Another important target organ in ecotoxicology that is not covered elsewhere in this text is the respiratory organ of nonmammalian aquatic vertebrates and many invertebrates, the gill; gills of fishes have received the most attention as targets of toxicants. The gill epithelium is the major site of gas exchange, ionic regulation, acid–base balance, and nitrogenous waste excretions for fishes and other aquatic animals (Evans, 1987). Gills are immersed in a major exposure medium for these animals (surface water), so metabolically active epithelial cells are in direct contact with this medium. They also receive blood supply directly from the heart, through the ventral aorta. Thus, it is not surprising that gills comprise a very important target for many environmental pollutants, due to their critical physiological functions, central position in blood circulation pathways, and intimate relationship with the environment. The basic structure of fish gills is composed of branchial arches from which extend numerous filaments; from the filaments extend the lamellae (Wendelaar Bonga and Lock, in press). The lamellae are covered by a layer of epithelial cells that function in gas exchange, whereas the filament epithelium is dominated by other cell types, including pavement cells, mucous cells, and chloride cells; chloride cells are the primary location for ATPase activity and ion channels involved in ion transport.

Common structural lesions in gills caused by a diverse array of chemicals include cell death (via necrosis and apoptosis), rupture of the epithelium, hyperplasia and hypotrophy of various cell populations that can lead to lamellar fusion, epithelial swelling, and lifting of the respiratory epithelium from the underlying tissue (Wendelaar Bonga and Lock, in press). Chloride cells have received particular attention due to their key role in ionic homeostasis. For example, metals such as cadmium, copper, lead, silver, and zinc have been shown to interfere with their function in ion transport. In some cases, this may be due to inhibition of ATPase activities and/or increased membrane permeability (Spry and Wood, 1985; Wendelaar Bonga and Lock, 1992; Li *et al.*, 1998; Rogers *et al.*, 2003; Bury, 2005). The stress response, which results in elevated blood concentrations of epinephrine and cortisol, and associated responses such as increased cardiac output and elevated blood pressure, can also perturb ionic balance by promoting passive loss of ions such as Na^+ and Cl^- (McDonald and Milligan, 1997). A variety of contaminants have been shown to evoke the stress response in fish, sometimes concomitantly with perturbations in ionic balance (Hontela, 1997; Webb and Wood, 1998; Chowdhury *et al.*, 2004). Also, gill damage appears to be the primary cause of the acute toxicity of PAH-mediated phototoxicity in fish (see the section "Oxidative Stress"). Weinstein *et al.* (1997) reported histopathological impacts of UV + fluoranthene in gills of fathead minnows including severe damage to mucosal cells, inflammation, and apparent accumulation of lipid peroxidation products; these effects likely resulted in respiratory stress, and lethality. This study elegantly demonstrates a progression from biochemical mechanism (oxidative stress) to target organ damage (gill respiration) to an important organismal impact (death). Mortality and important sublethal organismal impacts that have received substantial attention among ecotoxicologists comprise the following section.

Organismal Effects

Mortality In similarity with impacts on human health, chemical pollution of the environment does not in most cases attain levels sufficient to outright kill wildlife. Concern in the ecotoxicological context is overall more for long-term, chronic impacts on organismal variables such as reproduction and development, behavior, and disease susceptibility, and how such impacts parlay into impacts at population and higher levels of organization. However, numerous cases of wildlife mortalities (particularly birds) due to exposures to chemical pollution have been observed, including cases associated with chronic oil discharges (Wiese and Robertson, 2004) and major oil releases from events such as the Exxon Valdez tanker wreck in Alaska (Peterson *et al.*, 2003) and the 1991 Gulf War (Evans *et al.*, 1993), lead from spent shot (Clark and Scheuhammer, 2003) and mines (Henny, 2003), and pesticide exposures (Mineau *et al.*, 1999). While not a direct toxic chemical effect, hypoxia can be an important cause of fish and invertebrate mortality in aquatic systems; anthropogenic inputs of nutrients associated with sewage or fertilizers that enhance the growth of phytoplankton can cause or exacerbate hypoxia (Paerl *et al.*, 1999; Wu, 2002). While direct mortality may not be a commonplace effect of toxic chemicals in natural systems, mortality comprises a major end point in toxicity testing, discussed later.

Reproduction and Development Impacts on reproduction and development comprise perhaps the greatest concern among potential sublethal effects of xenobiotics on animals inhabiting natural systems. This is due to sensitivities of the physiological processes involved that have been described for a number of pollutants, and the importance of reproduction and development to population dynamics, a key ecological concern. Moreover, the discovery of the effects of some organochlorine insecticides on avian reproduction (particularly eggshell thinning) and resulting population crashes of several predatory bird species, and the public's awareness through the publication of Carson's *Silent Spring* in 1962, can be associated with the birth of ecotoxicology. Concern for reproductive and developmental effects has blossomed in recent years, with the widespread detection of endocrine disruptors in the environment.

A variety of environmental contaminants have been associated with reproductive and/or developmental effects in wildlife populations, with this association supported by controlled laboratory studies. Chlorinated hydrocarbons have continued to generate concerns, although many (DDT and other insecticides, and PCBs) have had their production and use sharply curtailed. For example, DDT, its major metabolite in birds (DDE), and PCBs have been associated with reproductive and developmental impacts in bird populations in the Great Lakes, southern California, the Puget Sound, and the Arctic (Fry, 1995; Custer *et al.*, 1999; Bustnes *et al.*, 2005). Also, alligators (*Alligator mississippiensis*) inhabiting a DDT-polluted lake in Florida have exhibited reproductive and developmental perturbations (Guillette *et al.*, 2000). Evidence also indicates that PCBs impact marine mammal reproduction, including that of bottlenose dolphins (*Tursiops truncates*) (Schwacke *et al.*, 2002; Wells *et al.*, 2005).

Deleterious impacts on fish reproduction have been associated with environmental exposures to a number of contaminants. Case studies include PAHs (and other chemicals) accumulated in sediments in urban areas and harbors in the Puget Sound (Johnson *et al.*, 1993) and northeastern United States (Johnson *et al.*, 1994) or associated with oil spills such as the Exxon Valdez (Sol *et al.*, 2000). Other examples include effluents from bleached paper mills in various locations, including Canada (Munkittrick *et al.*, 1991), and selenium, for example, emanating from coal-fired power plant fly ash stored near freshwater lakes (Lemly, 2002) or in streams due to coal mine runoff (Holm *et al.*, 2005). Notably, selenium produced severe developmental effects in water birds feeding in a created wetland in central California (Kesterson National Wildlife Refuge) that concentrated naturally occurring selenium (Ohlendorf, 2002). Additionally, severe developmental anomalies have also been observed in natural populations of marine gastropods exposed to tributyltin (TBT), which has been used extensively as an antifouling paint on ship hulls (Ruiz *et al.*, 2005).

The developmental effects of dioxins (TCDD) and coplanar PCBs on vertebrate development have received substantial attention. To some extent, this work was motivated by analyses that indicated that these compounds were responsible for population crashes of Great Lakes' fisheries (particularly lake trout, *Salvelinus namaycush*, in Lake Ontario) in the 1950s and 1960s, as well as for developmental impacts on other wildlife, particularly piscivorous birds and mammals in the region (Gilbertson *et al.*, 1991; Cook *et al.*, 2003). Laboratory investigations, largely with fish and bird models, have shown that embryo development is very sensitive to these compounds, and such effects likely underlaid the population crashes (Fairbrother *et al.*, 1999; Cook *et al.*, 2003). These investigations have included elegant mechanistic studies that revealed cardiac development was particularly sensitive to these chemicals, and concluded that developmental perturbations are largely receptor mediated, that is, they are dependent on binding of the chemical (such as TCDD) with the AHR described above (Hankinson, 1995; Heid *et al.*, 2001; Tanguay *et al.*, 2003; Antkiewicz *et al.*, 2005). Much of the more recent work in this area has been done with zebrafish (*D. rerio*), a powerful model for molecular and developmental toxicology due to ease of visually examining development through a clear chorion, rapid development (approximately four days from fertilization to hatch), and abundant genetic information, including gene sequences (Carney *et al.*, 2006). For example, with a known gene sequence, one can design morpholinos to block translation of specific mRNAs; morpholinos are oligonucleotides with a modified "backbone" that renders them stable (resistant to DNA/RNAase activities) and thus able to transiently block translation of specific protein targets. Carney *et al.* (2004) employed morpholinos to knock down AHR translation in zebrafish embryos, which greatly reduced the developmental toxicity of TCDD, confirming the role of the AHR in dioxin toxicity. Morpholinos were also employed to investigate the role of CYP1A; for example, its upregulation via TCDD activation of the AHR could enhance oxidative stress. Teraoka *et al.* (2003) observed marked reductions of TCDD toxicity with either AHR or CYP1A morpholinos, while Carney *et al.* (2004) observed protection with the AHR but not the CYP1A morpholino. While the role of CYP1A in dioxin effects on development remains unclear, pathways downstream of the AHR other than CYP1A are likely involved.

Similar concerns have emerged for the developmental effects of PAHs, particularly in fish. Hydrocarbons, in large part PAHs, associated with oil spills, contaminated sediments, paper mill effluents, and creosote used for wood treatment have profound developmental effects in fish embryos (Billiard *et al.*, 1999; Carls *et al.*, 1999; Meyer *et al.*, 2002). In many cases, the effects observed visually appear similar to those observed in fish embryos exposed to dioxins and coplanar PCBs, and include malformed hearts ("tube heart"), craniofacial deformities, hemorrhaging, and edema of the pericardium and yolk sac, the latter resulting in a distended, faintly blue yolk sac and hence a name given to this syndrome—"blue sac disease" (Spitsbergen *et al.*, 1991). The mechanisms by which PAHs produce this effect are unresolved, and likely include more than a single mechanism—not surprising in light of the myriad of chemicals comprising hydrocarbon/PAH mixtures in the environment. In some cases, effects appear to be AHR-independent. Incardona *et al.* (2005) concluded in studies with zebrafish and employing morpholinos that in weathered crude oil, tricyclic PAHs (such as phenanthrene and dibenzothiophene [DBT], the latter a sulfur-substituted PAH) accounted for the bulk of cardiovascular teratogenesis, and rather than mediating toxicity, the AHR–CYP1A pathway afforded some protection. Wassenberg and Di Giulio (2004a) and Wassenberg *et al.* (2005) observed marked synergies in the developmental toxicity to killifish (*Fundulus heteroclitus*) embryos between higher molecular weight PAHs that are AHR agonists (BaP and BNF) and PAHs that inhibit CYP1A (α-naphthoflavone [ANF], fluoranthene, DBT, and carbazole—a nitrogen-substituted PAH). Also, ANF enhanced the toxicity of a water-based extract of sediments contaminated with weathered creosote (Wassenberg and Di Giulio, 2004b). In subsequent studies with zebrafish embryos investigating the synergistic toxicity of BNF and ANF, the AHR morpholino provided protection, while the CYP1A morpholino enhanced toxicity (Billiard *et al.*, 2006). Collectively, these PAH studies suggest that in the context of embryo toxicity, CYP1A plays a protective role, presumably by mediating metabolism and clearance of these metabolically labile compounds. This is in contrast to the metabolism-resistant dioxin-like compounds, where CYP1A either appears to play a role in mediating toxicity or has no effect. For some PAHs, such as lower molecular weight (tricyclic) PAHs that have little or no activity as AHR ligands, developmental toxicity appears AHR-independent, while the developmental toxicity of some higher molecular weight PAHs that are AHR agonists appears in part AHR-mediated. In addition, oxidative stress may play a role in the developmental toxicity of some PAHs to fish embryos (Bauder *et al.*, 2005). PAHs comprise a ubiquitous class of contaminants that appear to be generally increasing in the environment, reflecting urbanization, population growth, and use of fossil fuels (Van Metre and Mahler, 2005).

Contaminant effects on development are often difficult to discern in field studies, due to the small size of embryos and the fact that developmental impacts generally either are lethal or greatly reduced survival. However, early life stages of most organisms are generally more sensitive to xenobiotics than other life stages; thus, developmental impacts merit careful attention by ecotoxicologists.

Disease Susceptibility Disease plays an important role in regulating and sometimes seriously impacting populations of free-living organisms. Of great concern are interactions between disease organisms and environmental contaminants, particularly potential impacts of chemicals on immune systems that render organisms more susceptible to disease. The question is often raised about how chemical pollution elevates the role of disease in population viability and dynamics.

Both field observational and laboratory experimental studies motivate this concern. For example, forensic evidence suggested that pHAHs such as dioxins and PCBs may have played a role in mass mortalities of seals and other marine mammals in the Baltic Sea that were directly attributed to viral infections

(Ross *et al.*, 1996). Captive harbor seals (*Phoca vitulina*) fed fish from the Baltic Sea displayed a number of immune system deficits relative to seals fed fish from uncontaminated Atlantic Ocean sites, including impaired natural killer (NK) cell activity, in vitro T-lymphocyte function, antigen-specific in vitro lymphocyte proliferative responses, and in vivo delayed type hypersensitivity and antibody responses to ovalbumin. These effects were correlated with greater concentrations of TCDD equivalents in fish from the Baltic Sea. In a case–control study using long-term data from studies of marine mammal strandings in the United Kingdom, Hall *et al.* (2006) concluded that each 1 mg/kg increase in total PCB concentrations in blubber resulted in an average increase in mortality due to infectious disease of 2% in harbor porpoises (*Phocoena phocoena*). In a study of free-ranging logger-head sea turtles (*Caretta caretta*) collected in North Carolina, Keller *et al.* (2006) observed significant correlations between selected immune responses (lysozyme activity and lymphocyte proliferation) and concentrations of PCBs and chlorinated insecticides (DDE and chlordanes); these correlations were supported by in vitro studies with these chemicals in isolated turtle leukocytes. Similarly, Auffret *et al.* (2006) observed responses associated with immunosuppression in mussels (*Mytilus galloprovincialis*) that generally tracked chemical pollution gradients in the western Mediterranean Sea. Using available laboratory and field data, Loge *et al.* (2005) developed a model to assess the effects of environmental stressors, including chemicals, on disease susceptibility in migrant juvenile salmon in the Columbia River Basin, Washington. They concluded that chemical and nonchemical stressors contributed equally to disease-induced mortalities that were predicted to range from 3% to 18% of the population, depending on residence time.

Numerous laboratory studies have demonstrated chemical impacts on immune systems in animals of ecological relevance. These include effects of pesticides on amphibians (Christen *et al.*, 2004), PCBs on channel catfish (*Ictalurus punctatus*) (Rice and Schlenk, 1995), heavy metals on rainbow trout (Sanchez-Dardon *et al.*, 1999), PAHs on bivalves (Wootton *et al.*, 2003), and flame retardants (polybrominated diphenyl ethers [PBDEs]) on American kestrels (*Falco sparverius*) (Fernie *et al.*, 2005). Fairbrother *et al.* (2004) reviewed the literature concerning effects of chemicals on immune systems of birds, emphasizing potential impacts on wildlife species, and Zelikoff *et al.* (2002) performed a similar review for fish. The potential effects of chemicals on immune function and disease susceptibility in wildlife is clearly a very important subject in ecotoxicology and one likely to see significant advances in the near future as powerful genomic tools become more available for representative species.

Behavior The impacts of chemicals on animal behavior have received significant attention among ecotoxicologists. Relatively subtle effects on behaviors associated with, for example, mating and reproduction, foraging, predator–prey interactions, preference/avoidance of contaminated areas, and migration have potentially important ramifications for population dynamics. However, difficulties in objective quantifications of behaviors and laboratory to field extrapolations appear to have limited the application of this area to ERAs, and by extension, perhaps to funds available for basic research. In some cases, however, biochemical mechanisms underlying behavioral effects have been elucidated that may assist with these issues and provide useful biomarkers for behavioral toxicants in field studies.

As noted by Rand (1985), chemicals causing behavioral effects in wildlife are often known from mammalian studies to be neurotoxicants. For example, in an early study, Grue *et al.* (1982) noted reduced nest attentiveness in female starlings dosed with the AChE-inhibiting organophosphate insecticide dicrotophos; this study took advantage of the relative ease of attracting wild starlings to artificial nest boxes that is advantageous for detailed studies, a phenomenon that has been employed in subsequent avian ecotoxicological studies (Parker and Goldstein, 2000). Grue *et al.* (1997) and Walker (2003) reviewed the behavioral effects in birds of these and other neurotoxic insecticides.

Behavioral effects of insecticides have also been observed in fish. For example, Scholz *et al.* (2000) reported adverse impacts of the organophosphate diazinon on olfactory-mediated behaviors such as the alarm response and homing in the Chinook salmon (*Oncorhynchus tshawytscha*), and Sandahl *et al.* (2005) observed similar thresholds for the effects of another organophosphate (chlorpyrifos) on swimming and feeding behaviors and on AChE inhibition in coho salmon (*O. kistich*). The effects of pollutants, including pesticides, on fish behavior were reviewed by Scott and Sloman (2004).

Mercury, particularly as methylmercury, comprises another potent neurotoxin that has been shown to perturb behavior in wildlife. For example, golden shiners (*Notemigonus crysoleucas*) fed diets containing methylmercury that resulted in tissue mercury concentrations consistent with those observed in this species in northern US lakes exhibited perturbed predator avoidance behaviors (Webber and Haines, 2003). In a study employing fish captured in the field and brought into the laboratory for behavioral analysis, Smith and Weis (1997) observed that killifish captured from a mercury-polluted tidal creek in New Jersey exhibited reduced feeding activity and greater mortality due to predation than killifish from an uncontaminated site. Using mercury concentrations in feathers as a marker for exposure, Heath and Frederick (2005) observed a negative correlation between mercury exposure and nesting activity among White Ibises (*Eudocimus albus*) in the Florida Everglades that may be related to behavioral effects. In studies with wild mink (*Mustela vision*) collected in Canada, Basu *et al.* (2005) observed significant correlations between mercury concentrations in brains and densities of neurochemical receptors (cholinergic and dopaminergic) associated with animal behavior. The effects of mercury on wildlife, including behavioral impacts, were reviewed by Wolfe *et al.* (1998).

Environmental contaminants not generally thought of as neurotoxicants have also been shown to perturb behavior. For example, cadmium and copper have been shown to impact olfactory neurons and associated behaviors (preference/avoidance to chemicals, including pheromones) in several fish species (Saucier *et al.*, 1991; Baker and Montgomery, 2001; Baldwin *et al.*, 2003). Copper exposure in zebrafish also led to loss of neurons in the peripheral mechanosensory system ("lateral line"), which could lead to altered behaviors associated with schooling, predator avoidance, and rheotaxis (physical alignment of fish in a current) (Linbo *et al.*, 2006). Carvalho and Tillitt (2004) reported loss of retinal ganglion cells in rainbow trout exposed to TCDD; these cells link the eye with the brain, and in this study deficits in visual acuity and prey capture rates were noted in TCDD-exposed fish. Clearly, numerous mechanisms of chemical toxicity can result in behavioral impacts, including direct toxicity to neurons, alterations in hormones that modulate behaviors, and impaired energy metabolism. In some cases, impaired behavior may comprise a sublethal impact with substantive ecological consequence (Scott and Sloman, 2004).

Cancer Beginning in the 1960s, numerous cases of cancer epizootics in wildlife that are associated with chemical pollution,

particularly in specific fish populations, have been reported in North America and northern Europe (Harshbarger and Clark; 1990; Vethaak, 1992). As in humans, cancer in these animals occurs largely in relatively older age classes and therefore is oftentimes considered a disease unlikely to directly impact population dynamics or other ecological parameters. However, this may not always be the case, particularly in species that require many years to attain sexual maturity and/or have low reproductive rates. In any event, the occurrence of high incidences of cancer in wildlife populations raises serious concerns for environmental quality at those locations experiencing these epizootics. For these reasons, as well as for concerns for human health in these areas, and the advantages of alternative models such as fish for understanding chemical carcinogenesis, these epizootics have motivated substantial research in several areas relevant to human health and ecotoxicology.

In field studies of cancer outbreaks in aquatic and marine systems, typically only selected species exhibit elevated cancer rates associated with chemical contamination. A major contributor to this differential cancer susceptibility in wild fish populations is clearly lifestyle; benthic (bottom-dwelling) species such as brown bullhead (*Ameriurus nebulosus*) and white sucker (*Catostomus commersoni*) in freshwater systems, and English sole (*Parophrys vetulus*) and winter flounder (*P. americanus*) in marine systems generally exhibit the highest cancer rates in polluted systems (Baumann, 1998). The bulk of chemicals in these systems associated with cancer epizootics, such as PAHs, PCBs, and other halogenated compounds, reside in sediments; benthic fish live in contact with these sediments and prey in large measure on other benthic organisms. Thus, benthic fish experience greater exposures to carcinogens than other species in these systems. Inherent biological differences may also play a role in species susceptibilities to chemical carcinogenesis; for example, laboratory studies have revealed marked differences among fish species in their abilities to activate PAH procarcinogens to DNA adduct-forming metabolite as well as to detoxify them through phase II metabolism (Collier *et al.*, 1992; Hasspieler *et al.*, 1994; Ploch *et al.*, 1998).

In their analysis of cancer epizootics in fish, Harshbarger and Clark (1990) concluded that cancers of the liver (hepatocellular neoplasms) had the strongest associations with chemical pollution, although cancers have been observed in other tissues in wild fish as well (Ostrander and Rotchell, 2005). PAHs appear to be the most implicated class of carcinogens associated with liver neoplasms in fish cancer epizootics. Studies implicating a key role for PAHs (and key PAH sources) include English sole in the Puget Sound (various urban and industrial sources; Malins *et al.*, 1987), brown bullhead in the Black River, Ohio (a coal-coking facility; Baumann and Harshbarger, 1998), and in the Potomac River watershed near Washington, DC (various point and nonpoint discharges; Pinkney *et al.*, 2001), and killifish in the Elizabeth River, Virginia (a wood treatment plant using creosote; Vogelbein *et al.*, 1990). As stated earlier, PAHs appear to comprise a class of contaminant generally increasing in the environment. The metabolism of PAHs such as BaP to reactive metabolites that form DNA adducts that initiates carcinogenesis, or conversely to excretable conjugates, was described earlier. It is noteworthy that the molecular and biochemical pathways underlying chemical carcinogenesis, such as PAH metabolism, DNA damage, and effects on oncogenes, are qualitatively similar between most fish and mammalian species examined.

This recognition of shared pathways has in part contributed to the use of various fish models for studying chemical carcinogenesis from a human health as well as from a broader environmental standpoint. An important historical event was the identification in Italy, France, and the United States during the 1950s and 1960s of aflatoxin as a potent liver carcinogen in farm-raised rainbow trout (Sinnhuber *et al.*, 1977). Subsequently aflatoxin, a fungal toxin produced by *Aspergillus flavus* that is of concern where grains and nuts are stored in wet conditions, was found to be carcinogenic to mammals including humans. Thus, the rainbow trout observations led to the discovery of a new and important class of chemical carcinogens, and the recognition that fish can be very sensitive to chemical carcinogenesis. Since that time, other fish species have been employed for laboratory studies related to chemical carcinogenesis, particularly medaka (*O. latipes*) and platyfish/swordtails hybrids (*Xiphophorus* spp.); zebrafish also show promise as a laboratory model (Ostrander and Rotchell, 2005). Compared with rodent models, fish models have advantages of reduced costs for propagation and housing, briefer time intervals between exposures and the expression of tissue changes indicative of carcinogenesis, and greater feasibility of performing large-scale studies with many animals to quantify dose–response relationships.

It is noteworthy that the great bulk of reports of elevated cancer rates in free-living animals occur in fish, with few reports of potentially chemically related cancers to our knowledge in other vertebrates. California sea lions (*Zalophus californianus*) stranded along the central California coast were found to have elevated cancer rates (18%), and concentrations of DDT and PCBs were greater in animals with cancer versus those determined to die of other causes (Ylitalo *et al.*, 2005). Martineau *et al.* (2002) reported elevated cancer rates (also 18%) in carcasses of beluga whales (*Delphinapterus leucas*) stranded along the shores of the St. Lawrence River estuary in Quebec, a system with elevated levels of PAHs. The authors noted that beluga was the only species of marine mammal among 20 inhabiting this system that exhibited elevated cancer rates, and that cancers are rare worldwide in marine mammals. It is likely that elevated exposures play an important role in the relatively high frequency of reports of cancers in benthic fishes; relative inherent sensitivities among mammals, birds, reptiles, amphibians, and fishes are unclear.

Population

A population is a collection of individuals of the same species that occupy the same space and within which genetic information can be exchanged. The study of populations is a central theme in ecological sciences and ecotoxicology is no exception. Assessment of toxicant effects on populations has been important in ecotoxicology since its inception (Newman, 2001). A well known, early instance is the sharp drop and then slow recovery of coastal populations of osprey (Spitzer *et al.*, 1978) and brown pelican (Anderson *et al.*, 1975) that occurred as a consequence of widespread DDT and DDE spraying and eventual banning. Another was the enhanced, genetically based tolerance of pest insect populations chronically sprayed with pesticides (Mallet, 1989). Industrial melanism, the premier example in biology textbooks of natural selection in wild populations, is another example of population ecotoxicology (Newman, 2001). Population ecotoxicology covers a wide range of topics with core research themes being (1) epidemiology of chemical related disease, (2) effects on general population qualities including demographics and persistence, and (3) population genetics.

The level of belief warranted for possible contaminant-related effects in nonhuman populations is assessed by applying routine epidemiological methods. Many methods described in epidemiology textbooks (Anders, 1993; Woodward, 2005) are applied to

Table 30-1

A Summary of One Popular Set of Rules of Thumb (Data from Fox, 1991) for Assessing Plausibility of a Causal Association in an Ecological Epidemiology

RULE	DESCRIPTION
1. Strength of association	How strong the association is between the possible cause and the effect, for example, a very large relative risk
2. Consistency of association	How consistently is there an association between the possible cause and the effect, for example, consistent among several studies with different circumstances
3. Predictive performance	How good is the prediction of effect made from the presence/level of the possible cause
4. Monotonic trend	How consistent is the association between possible cause and effect to a monotonic trend (ie, either a consistent increase or decrease in effect level/prevalence with an increase in exposure)
5. Inconsistent temporal sequence	The effect, or elevated level of effect, occurs before exposure to the hypothesized cause
6. Factual implausibility	The hypothesized association is implausible given existing knowledge
7. Inconsistency with replication	Very poor reproducibility of association during repeated field assessments encompassing different circumstances or repeated formal laboratory testing

NOTE: According to Fox, the first four rules are most useful in supporting a causal hypothesis if found to be true (ie, very strong, consistent, predictive, or monotonic association). The others are most useful for lessening belief in the causal hypothesis if true.

nonhuman populations, although with a slightly different balance because much more experimental exposure data are potentially available for nonhuman populations than for human populations. Rules of thumb for gauging the level of belief warranted by evidence that emerged from human epidemiology are also applied in population ecotoxicology. Hill's nine aspects of human disease association (Hill, 1965) might be used directly or after minor modification. As an example, Fox (1991) (Table 30-1) modified such rules of thumb to accommodate slight differences in the subject matter and approaches in population ecotoxicology. Conventional epidemiological descriptors and models are also applied. For example, Horness *et al.* (1998) quantified prevalence and relative risks for neoplastic liver lesions in English sole inhabiting areas with different sediment concentrations of PAHs. Logistic regression models were also used to identify relationships between these lesions and chemical and biological risk factors (Myers *et al.*, 1994).

Defining and predicting alterations in population size, dynamics, and demographic composition due to toxicant exposure has always been central in ecotoxicology and has become increasingly so in the last 15 years as regulatory agencies such as the US Environmental Protection Agency clearly reinforced their long-standing commitment to understanding chemical exposure effects on natural population viability.

Protecting populations is an explicitly stated goal of several Congressional and Agency mandates and regulations. Thus it is important that ecological risk assessment guidelines focus upon protection and management at the population, community, and ecosystem levels....

Environmental Protection Agency (1991)

Ecological theory and research (eg, Forbes and Calow, 1999) also indicate that metrics of effect to individuals are not especially good metrics of toxicant exposure effects to populations.

Models of exposed population dynamics suggest that reductions on population densities are not the only important changes brought about by chemical exposure. Some species populations fluctuate within a range of densities. These fluctuations are characteristic of the species strategy for maintaining itself in various types of habitats and toxicant exposure could potentially change this range (Simkiss *et al.*, 1993). Combined with decreases in population densities driven by external forces such as weather events, these toxicant induced modifications of the average population densities and dynamics can increase the risk of a population's density falling so low that local extinction occurs (Newman, 1995).

Demographic qualities can change with toxicant exposure in ways that influence the risk of local population extinction. Toxicants can change a species population's vital rates, that is, age- and sex-dependent death, birth, maturation, and migration rates, in complex ways. These changes in combination determine the population density and distribution of individuals among ages and sexes during exposure. The population's ability to resist external forces that reduce its size is determined by these demographic features (Gard, 1992; Sherratt *et al.*, 1999; Kammenga and Laskowski, 2000; Aubone, 2004). Consequently, considerable research effort is being spent on demographic methods for predicting exposed population changes and risks of extinction. AQ1

Demography explores vital rates of populations composed of individuals that differ in age and sex. Individuals in field populations can also differ in their spatial distribution and this influences the impact of toxicants (Newman, 2001). Individuals of the same species often are grouped into subpopulations within a habitat and all of these subpopulations together comprise a metapopulation (Fig. 30-9). Subpopulations in the metapopulation have different levels of exchange and different vital rates that depend on the nature of their habitat. Spatial distances and obstacles or corridors for migration influence migration among patches: habitat quality determines vital rates. An inferior habitat, such as a grossly contaminated one, can act as a sink into which individuals migrate from nearby superior (source) habitat. Migration can rescue a subpopulation or reduce its risk of local extinction. An individual migrating from the contaminated habitat to an uncontaminated one can express an adverse effect despite its present distance from the contamination, that is, the action-at-distance hypothesis of ecotoxicology (Spromberg *et al.*, 1998). The viability of the metapopulation can also be as strongly influenced by maintaining important migration corridors among subpopulations and protecting high-quality habitats (ie, keystone habitats) as by the general level of contamination within the metapopulation's habitat (Mauer and Holt, 1996; O'Connor, 1996; Spromberg *et al.*, 1998; Newman, 2001). AQ1

The genetics of exposed populations are studied to understand changes in tolerance to toxicants and to document toxicant influence on field populations. The capacity of some populations to become more tolerant of toxicants via selection is well documented. A few examples include increased tolerance of pine mice to endrin (Webb and Horsfall, 1967) and rats to warfarin (Partridge, 1979) after years

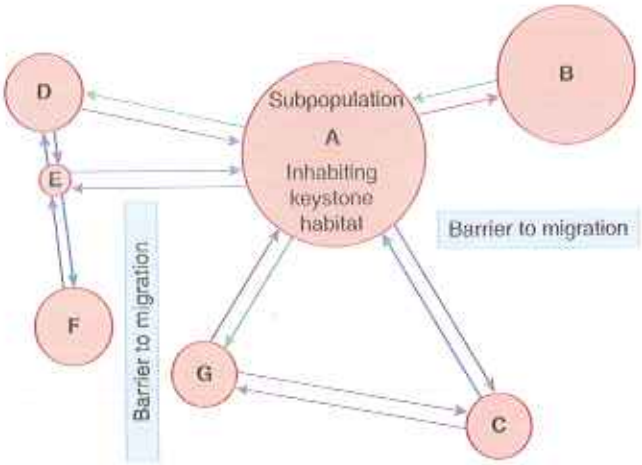

Figure 30-9. *Metapopulations are composed of subpopulations that differ in their vital rates and tendency to exchange individuals.* In this illustration, subpopulation A occupies a keystone habitat. The loss of subpopulation A would devastate the metapopulation. Also, loss of the migration corridor between subpopulations A, B, and D would devastate the metapopulation. In contrast, the loss of subpopulation F would not influence the metapopulation to the same degree.

of application of these agents for rodent control. More recently, Ownby *et al.* (2002) documented enhanced tolerance in populations of an estuarine fish chronically exposed to PAHs. Although genetically based increases in tolerance are well documented in wild populations, many exposed populations probably find themselves in situations in which they cannot adapt adequately because their genetic resources, nature of the toxicant, or spatial/temporal context within which the enhanced tolerance must evolve are inadequate. Also, in an ecotoxicological sense, all cases of increased tolerance do not fit the conventional context manifested in the examples just given. Some do not involve suborganismal changes to biochemical or anatomical features resulting in enhanced tolerance. For example, industrial melanism increases a peppered moth's fitness in the presence of soot by reducing its likelihood of being taken by a predator. The dominant light form of this moth has a lowered fitness in the presence of dark soot, and genetically based changes in color increase its fitness. The selective mechanism here is ecological, that is, fitness relative to avoiding visual predators.

AQ3

Genetic qualities are also used to infer past toxicant influence in an exposed population. For example, Mulvey *et al.* (2002, 2003) showed distinct genetic qualities in estuarine fish populations exposed to high concentrations of PAHs. Another piece of evidence demonstrating past toxicant influence on populations can be a change in genetic diversity. A drop in genetic diversity in populations is thought to be an adverse effect because genetic diversity is required in populations to evolutionarily adapt to environmental changes. Toxicants can influence genetic diversity by purely stochastic means. Genes can be lost in the population if the population is so drastically reduced in size that the chance of a rare gene being lost between generations becomes very high. Also, the average rate at which the frequency of a rare gene decreases through time due to genetic drift increases as the effective population size decreases. The effective population is the number of individuals contributing genes to the next generation so toxicant-related changes in demographic qualities can also accelerate genetic drift.

Community

An ecological community is an interacting assemblage of species populations occupying a defined habitat at a particular time. Populations in a community interact in many ways and, because these many interactions are complex, a community has properties that are not predictable from those of its component populations. Some species have such a crucial role (keystone species) or numerical dominance (dominants) that they are essential to maintaining community structure.[2] Other species contribute to the nature of the community in more subtle ways.

Ambiguity exists about the importance of all the species in a community relative to maintaining overall structure and balancing essential functions such as nutrient cycling, primary productivity, community respiration, and detritus processing. The redundant species hypothesis suggests that species function redundantly: if a species were lost, another with a similar function would increase in numbers to compensate. Only certain critical species such as dominant or keystone species are essential to the community. The rivet popper hypothesis suggests otherwise. Each species in a community is similar to one of the many rivets holding an airplane fuselage together. Each lost rivet contributes to a gradual weakening of the fuselage that will lead eventually to a failure in function. By analogy, each species disappearance diminishes a community's functioning.

Ecotoxicologists remain divided about which hypothesis is most relevant. Pratt and Cairns (1996) argue from evidence and a conservative stance that the rivet popper is the most appropriate. Ecotoxicologists and regulators who pragmatically set standards based on concentrations that will not harm more than a specified percentage of species in a community (eg, Stephan *et al.*, 1985) assume that the redundant species hypothesis is more pertinent. Although the redundant species hypothesis is assumed to be correct in many ERAs (Solomon and Sibley, 2002), recent theory (Loreau, 2004), modeling (Naeem *et al.*, 1994), and experimental evidence (Tilman, 1996; Tilman *et al.*, 1996; Salminen *et al.*, 2001) seem to support the rivet popper hypothesis. Biodiversity tends to foster community stability and function.

Communities take on characteristic structures as predicted by the Law of Frequencies: the number of individual organisms in a community is related by some function to the number of species in the community (Fig. 30-10). Ecotoxicants[3] can alter the resulting community structure in predictable ways by either directly impacting the fitness of individuals in populations that make up the community or altering population interactions. Community ecotoxicologists spend considerable effort trying to understand and predict ecotoxicant influences on community structure and essential functions.

Direct effects involve removal of a population or metapopulation from the community by reducing the Darwinian fitness of individuals enough that the population falls below some critical minimum size. Indirect effects can involve interference with

[2] In its most rudimentary context, community structure refers to the number of species present and the numbers of individuals present in each of these species. It can also refer to the distribution of species among different functional groups such as decomposers, detritivores, primary producers, primary consumers such as herbivores, secondary consumers such as carnivores that consume herbivores, etc.

[3] The conventional context for the term toxicant becomes difficult to retain without some qualification when dealing with populations, communities, and other higher level entities because an agent does not necessarily have to directly interact with the individual in order to harm it. As an example, an agent might eliminate a prey species, leading indirectly to the disappearance of a predator species that depended on it for sustenance. The "toxicant" did not poison the predator, yet it caused its demise nonetheless. A distinct term, ecotoxicant, is often applied to avoid confusion in such cases.

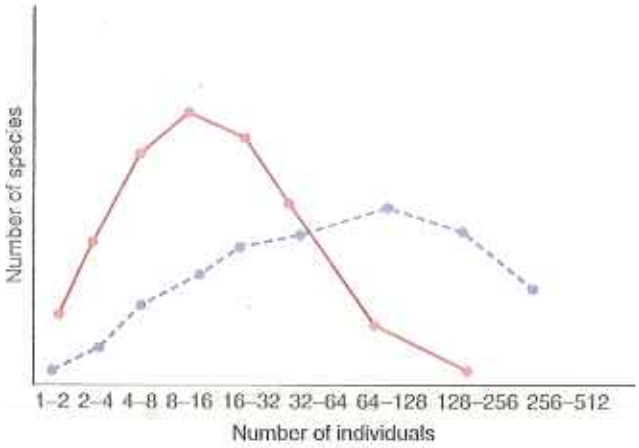

Figure 30-10. *Log-normal model of species abundance for an unexposed (solid line and red points) and toxicant-exposed community (dashed line and blue points).* Communities have distinct structure as shown here with the typical log-normal species abundance model. As first described by data from Patrick (1973), ecotoxicants tend to lower the mode of the species abundance curve and stretch the right tail outward. Ecotoxicants result in fewer intermediate abundance species and more extremely abundant species.

interspecies competition, predator–prey interactions, host–disease/parasite interactions, or symbiotic relationships such as pollination. The simplest competition model (Lotka–Volterra model) can be used to illustrate the potential for both direct and indirect effects on populations:

$$\frac{dN_1}{dt} = r_1 N_1 \left[1 - \frac{N_1}{K_1} - \frac{\alpha_{12} N_2}{K_1} \right],$$

$$\frac{dN_2}{dt} = r_2 N_2 \left[1 - \frac{N_2}{K_2} - \frac{\alpha_{21} N_1}{K_2} \right],$$

where N_1 and N_2 are the population sizes of competitors 1 and 2, K_1 and K_2 the carrying capacities of the environment for competitors 1 and 2, r_1 and r_2 the intrinsic rate of population increase (ie, birth rate – death rate) for competitors 1 and 2, α_{12} the competition coefficient quantifying the impact of the presence of competitor 2 on competitor 1, and α_{21} the impact of competitor 1 on competitor 2. Not only can exposure directly impact birth rates, death rates, and carrying capacity of each species, but it can also influence species persistence by shifting competition coefficients in favor of another species. Mathematically, it can be shown that the two competitors depicted in the Lotka–Volterra model can coexist only if two conditions are met, $K_1 < K_2/\alpha_{21}$ and $K_2 < K_1/\alpha_{12}$. So, a population can be lost from a community as readily by changing its competitive interactions as by directly changing its death and reproductive rates. Similar statements can be made about changes in predator–prey, host–disease, and various symbiotic interactions. As an example, concern expressed recently about unintended pesticide reductions in the number and diversity of pollinators in European farmlands (Newman *et al.*, 2006) could be partially responsible for the recently reported decline in insect-pollinated plant species in Britain and The Netherlands (Biesmeijer *et al.*, 2006). In another instance, reduced habitat cover and insect densities in European farmlands has had a significant impact on grey partridge populations (Rands, 1985; Chiverton, 1999). As another and final example involving predator–prey interactions, amphibian tadpole exposure to endosulfan increases the risk of predation by dragonfly larvae (Broomhall, 2002). None of these examples involves a direct poisoning by a toxicant, but instead, involves an ecotoxicant that adversely modifies species interactions.

Structural changes to communities can be detected in species abundance plots (see Fig. 30-10) or shifts in conventional community metrics calculated from community samples taken in the proximity of contaminated sites. Common metrics for species richness, diversity, and evenness are used to express changes in biodiversity. Richness is simply the number of species in the sampled community, or if a relative number of species in different communities is all that is needed, the number of species expected in a specified sample size such as a rarefaction richness estimate of 12 species in a sample of 100 individuals from a community. Evenness is a measure of how equitably the individuals in a community are spread among the species. Finally, diversity (heterogeneity) indices combine the elements of richness and evenness into one number. Generally, but not always, ecotoxicants lower species richness, evenness, and overall diversity. The regulatory premise is that these changes reflect a diminished community.

Recently, structural and functional qualities in communities have been combined to generate multimetric indices such as the Biotic Index of Integrity (IBI) (Karr, 1991). Ecological insight is used to select and then numerically combine community qualities such as species richness, health of individual animals in a sample, and the number of individuals in a sample belonging to a particular functional group, such as number of piscivorous fish. The IBI score for a study site is calculated and compared with that expected for an unimpacted site in order to estimate its biological integrity.

Another central theme in community ecotoxicology is toxicant transfer during trophic interactions. Toxicant concentrations can decrease (biodiminution), remain constant, or increase (biomagnification) with each trophic transfer within a food web. POPs with moderately high lipid solubility ($5 < \log K_{ow} < 7$ or 8; Thomann, 1989; Connell, 1990) and minimal metabolic breakdown in an organism can biomagnify to harmful concentrations. Metals that biomagnify are mercury and the alkali metals, cesium and rubidium. Zinc, an essential metal that is actively regulated in individuals, can exhibit biomagnification or biominification depending on whether ambient levels are below or above those required by the organism to function properly. Biominification is facilitated in a marine food web after sequestration in intracellular phosphate granules of molluscan prey species (Nott and Nicolaidou, 1993) and biomagnification by active regulation in zinc-deficient terrestrial communities (Beyer, 1986). The biomagnification of mercury is enhanced by its microbial transformation to methylmercury. Biomagnification of the potassium analogs, cesium and rubidium, is facilitated by the differences in their influxes and effluxes that favor retention in organisms (Rowan and Rasmussen, 1994; Campbell *et al.*, 2005). AQ4

Quantifying the trophic position of a species in a community is essential to modeling biomagnification. Most trophic systems are not simple "food chains." Most individuals in a community can feed on different species depending on their life stage, seasons, and relative abundances of prey species. These trophic interactions are best described as occurring in a trophic web, not a trophic chain.

Conveniently, trophic position of an individual within a complex food web can be quantified with nitrogen isotopes. Generally, ^{14}N passes through biochemical pathways faster than ^{15}N, resulting in excretion of waste with a slightly higher $^{14}N/^{15}N$ ratio than in ingested food. The relative amounts of ^{14}N and ^{15}N will be slightly biased toward the heavy isotope in tissues of a species relative to those of its food source(s). This discrimination between the heavy and light N isotopes continues through food webs, allowing the trophic position of each participating species to be estimated. The metric used for this purpose, the $\delta^{15}N$, expresses the quotient of

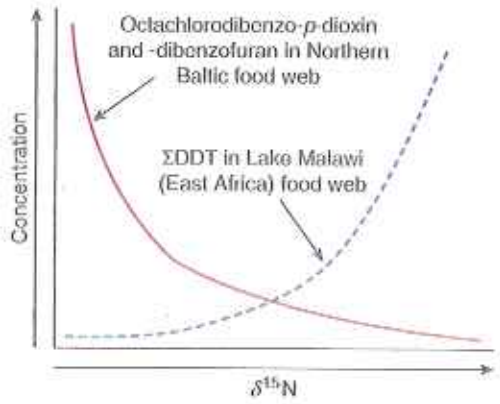

Figure 30-11. *Modeling ecotoxicant concentration versus trophic position as quantified with $\delta^{15}N$.* Power models were applied to octachlorodibenzo-*p*-dioxin/dibenzofuran concentrations in a North Baltic food chain (data from Broman *et al.*, 1992) and the ΣDDT (sum of primarily the *p,p'* isomers of DDD, DDE, and DDT) in food webs of Lake Malawi (data from Kidd *et al.*, 2001). Biomagnification and minification were evident for ΣDDT and octachlorodibenzo-*p*-dioxin/dibenzofuran, respectively.

these two isotopes in the biological tissue of interest relative to the quotient expected in the atmosphere:

$$\delta^{15}\mathrm{N} = 1000\left[\frac{[^{15}\mathrm{N}_{\mathrm{Tissue}}]/[^{14}\mathrm{N}_{\mathrm{Tissue}}]}{[^{15}\mathrm{N}_{\mathrm{Air}}]/[^{14}\mathrm{N}_{\mathrm{Air}}]} - 1\right].$$

The change in toxicant concentrations within food webs is modeled using the δ^{15}N, which quantifies trophic position of the species from which the tissue sample was taken (Fig. 30-11). Linear and exponential models are commonly applied:

$$\text{Concentration} = a + b\delta^{15}\mathrm{N},$$
$$\text{Concentration} = 10^{a+b\delta^{15}\mathrm{N}} \quad \text{or} \quad e^{a+b\delta^{15}\mathrm{N}}.$$

Ecosystem to Biosphere

Ecosystems are the functional unit of ecology composed of the ecological community and its abiotic habitat. Systems ecologists try to describe and predict energy and mass cycling in and flow from ecosystems. The ecotoxicologist's interest in ecosystems includes understanding how toxicants diminish an ecosystem's capacity to perform essential functions and to understand toxicant movement enough to assess exposure within different ecosystem components.

Many of the effects described above for exposed communities are relevant here. As an example, Allred and Giesy (1988) demonstrated that elevating cadmium concentrations in an artificial stream reduced decomposition rates of dead leaves. Odum (1985) suggested that other changes to be expected with increased ecosystem stress include an increased loss of nutrients, increased community respiration, and an imbalance of primary production and respiration.

Studies of toxicant movement within ecological systems are conducted at extremely different scales (Fig. 30-12). Conventional ecosystem studies involve descriptions of contaminant concentrations and movements in easily defined ecosystems such as lakes, forests, or fields. Some toxicants, especially those subject to wide dispersal by air or water, cannot be completely understood in this framework so a landscape scale might be chosen instead. As an example, acid precipitation might be examined in the context of an entire watershed, mountain range, or even a continental region. As another example (see Fig. 30-12, top right panel), the fish tissue concentrations of a PCB congener and other POPs were measured

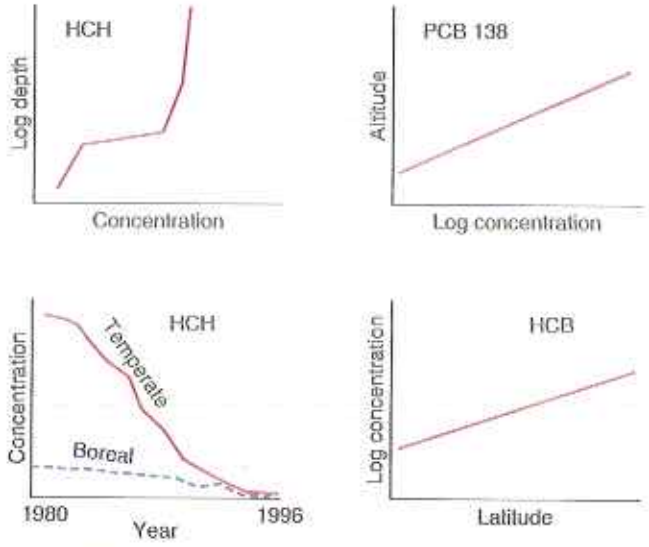

Figure 30-12. *Examples of scales relevant to assessments of ecotoxicant distributions.* The smallest scale example (top left) is the vertical distribution of α-hexachlorocyclohexane (HCH) in a stratified region of the Sea of Japan (data from Chernyak *et al.*, 1995). A slightly larger scale is reflected in the change in the polychlorinated biphenyl congener PCB 138 in tissues of fish inhabiting European lakes at different altitudes (top right panel, data from Fernandez and Grimalt, 2003). Representing a subcontinental scale study is the temporal change in atmospheric α-hexachlorocyclohexane concentrations in temperate and boreal regions of central northern Europe (bottom left panel, data from Wania *et al.*, 1999). The largest scale encompasses the entire earth, showing the influence of latitude on hexachlorobenzene (HCB) concentration in tree bark (bottom right panel, data from Simonich and Hites, 1995).

in high mountain region lakes of Europe. Concentrations for several were related to the altitude at which a lake sat. The relationships between altitude and concentrations of the various POPs was interpreted based on atmospheric movement of the POPs and each POP's propensity to either volatilize or condense at a particular altitude-dependent temperature regime. Still other ecotoxicants require a global context in order to fully understand their movements and accumulation. As an example, hexachlorobenzene concentration in tree bark collected worldwide showed a clear latitudinal gradient. Its global distribution and those of other sampled POPs were a function of their relative volatilities. The volatile hexachlorobenzene moved more readily toward the poles than less volatile POPs such as endosulfan and DDT. The differential global movement of POPs due to differences in volatility and partitioning behavior was called global distillation (Wania and Mackay, 1996). The balance between a POP's tendency to condense or evaporate at different latitude-related temperatures determines its atmospheric mobility and its ultimate global deposition pattern (Wania and Mackay, 1996).

APPROACHES

Many approaches have been developed to detect and quantify contaminant effects. These span the levels of organization described above. Approaches widely applied in ecotoxicology include standardized toxicity tests designed to meet regulatory needs and biomarkers for organismal exposure and effects. For higher levels, a range of ecological methods exists for population, community, and ecosystem effects. Other techniques such as geographic information system (GIS) analysis of impacts allow the ecotoxicologist to assess impact encompassing large spatial scales. These approaches and examples of their applications are described in this section.

Toxicity Tests

Toxicity testing encompassing representative animals and plants at different levels of organization offers a practical approach to characterize chemical effects on biological systems. While it is widely known that toxicity tests cannot mimic the complex interactions and variable conditions of natural ecosystems, they address the potential direct effects of toxic substances on individual ecosystem components in a controlled and reproducible manner. A number of testing guidelines have been put forth by regulatory bodies and organizations worldwide to meet requirements for chemical registration or authorization (OECD, 1981; MAFF, 1985; US EPA OPPTS, 1996a; ASTM International, 2006), with numerous subsequent revisions. Different sets of guidelines apply to specific countries, regions, or products, and can differ significantly in their requirements. The harmonized guidelines put forth by the US EPA Office of Prevention, Pesticides and Toxic Substances (US EPA OPPTS, 1996a) were created in an attempt to lessen variations in testing requirements, and bring together requirements from the Organisation for Economic Co-Operation and Development (OECD), the US EPA Office of Pollution Prevention and Toxics (OPPT), and the Office of Pesticide Programs (OPP).

Ecotoxicology tests feature a wide variety of aquatic (including algae, invertebrates, tadpoles, bivalves, shrimp, fish), avian (quail, duck), and terrestrial species (soil microorganisms, crops, honey bees, earthworms, wild mammals). Species are selected based on their traditional use as laboratory animals, but also on ecological relevance, which further complicates global harmonization of ecological testing. In addition, special considerations apply to testing of aquatic species due to the unmistakable differences in the way aquatic species are exposed to toxicants (US EPA OPPTS, 1996b). For instance, water quality monitoring and investigation of the solubility and stability of the test substance under the conditions of testing, along with determination of nominal versus measured concentrations, are common practices in aquatic toxicology. Testing can be conducted in aqueous systems without renewal of the test substance (static), renewal at predetermined time intervals (static-renewal), or continuous flow of test substance through the test compartment (flow-through).

Acute toxicity testing consists of single species exposed to various concentrations of the test substance. The most common end point in acute tests is death, although abnormal behavioral or other gross observations are commonly noted, and nonlethal end points occasionally apply (eg, immobilization for daphnids, shell deposition in oysters). Variations in acute toxicity studies comprise testing of different species (such as fresh vs saltwater fish, bobwhite quail vs mallard duck), life stages (embryo, larva, juvenile), environmental influences (eg, presence of organic material), or sediment exposures. Data from different test concentrations and time points are used to derive concentration–response curves and predicted values such as the LC_{50} (median lethal concentration), EC_{50} (median effective concentration), or IC_{50} (median inhibition concentration). The LC_{50} represents the concentration of test substance killing 50% of the tested animals and EC_{50} the concentration of test substance affecting 50% of the test population during a specified period of time, such as growth; the IC_{50} is the concentration causing a 50% reduction in a nonquantal measurement (such as movement) for the test population. More quantitative values derived from acute tests are the lowest observed effect concentration (LOEC), that is, the lowest concentration where an effect is observed, and the no observed effect concentration (NOEC), the highest concentration resulting in no adverse effects.

Short-term laboratory studies conducted with single species are useful for rapid screening, provide information on thresholds for effects and selective and comparative toxicity, and can be used as range finders to guide subsequent, often more involved studies. Long-term and reproductive studies evaluate the effects of substances on organisms over extended periods of time and/or sequential generations (chronic toxicity, life cycle, reproduction). End points include both quantal (such as mortality) and nonquantal (reproduction, growth) measurements, and can be used to derive additional values, other than previously mentioned in acute toxicity tests. These include the calculation of threshold values, such as the maximum acceptable toxicant concentration (MATC), which is the maximum chemical concentration not toxic to test organisms, and the BCF, which estimates the tissue concentration in relation to the average exposure concentration in the test medium (eg, water).

Unique to ecotoxicology are the more elaborate microcosm, mesocosm, and field studies. Microcosms are representative aquatic or terrestrial ecosystems created under laboratory conditions that include a number of relevant species (such as protozoa, plankton, algae, plants, invertebrates). Simulated field studies or mesocosms can be created in the laboratory or in the field (eg, artificial streams, ponds) or consist of enclosures of existing habitats, containing representative soil, water, and biota. Lastly, full-scale field studies (aquatic organisms, terrestrial wildlife, pollinators) evaluate the effects of a substance on wildlife under real-life scenarios of actual use conditions of a product (eg, pesticide field usage rate), and thus, are more complicated, subject to considerable variability, and require extensive background knowledge of the local population and community dynamics.

As a final point, plant studies are a significant component of ecological toxicity testing, particularly for pesticide registration, and involve tiered testing of both target area and nontarget terrestrial and aquatic plants. Target area plants are those that are present in the area where the substance will be routinely used (application area), but which are not anticipated to be affected. Nontarget plants are those outside of the intended use area. End points of phytotoxicity include seedling emergence and growth, vegetative vigor, and rhizobium–legume toxicity, among others, and central to the toxicity testing with plants are the substrate and environmental conditions, which greatly influence plant health.

Biomarkers

The National Academy of Sciences (1987) defined a biomarker as "a xenobiotically induced variation in cellular or biochemical components or processes, structures, or function that is measurable in a biological system or sample." In the context of ecotoxicology, this definition has been modified slightly to refer to biochemical physiological or histological indicators of either exposure to or effects of xenobiotic chemicals at the suborganismal or organismal level (Huggett *et al.*, 1992). The term is most often employed to refer to molecular, physiological, and organismal responses to contaminant exposure that can be quantified in organisms inhabiting or captured from natural systems. A response that is limited to laboratory studies falls outside the generally held concept of a biomarker.

By definition, biomarkers do not directly provide information concerning impacts on the higher levels of organization that ecotoxicology ultimately endeavors to discern. Nevertheless, biomarkers often provide important ancillary tools for discerning contaminant exposures and potential impacts of ecological importance. The development and use of biomarkers in ecotoxicology is motivated by several factors. These include the inherent instabilities of many contaminants (such as PAHs and many pesticides) that make

measures of exposure by direct tissue residue analysis difficult, the relative biological sensitivity of many biomarkers, the chemical specificity of some biomarkers that serve to contribute to the identification of chemicals having biological effects, and relatedly, the linkage of some biomarkers to underlying mechanisms of toxic action. Additionally, while populations and higher level effects are of greatest concern, variables associated with these levels are oftentimes relatively insensitive to chemicals and other stressors, take long periods of time to become manifest, and/or have difficult or imprecise methods for their analysis. Thus, biomarkers can provide sensitive early warning signals of incipient ecological damage (van der Oost *et al.*, 2003), in essence an ecological counterpart to the "canary in the coal mine" approach for preventing harm to coal miners. However, biomarkers do not provide adequate standalone data in the context of ecological assessments of contaminant effects. At this time and for the foreseeable future, such assessments generally involve a "weight of evidence approach," coalescing information obtained from chemical analyses, toxicity tests, biomarkers, and ecological indicators (sometimes referred to as "bioindicators").

In earlier discussions, a number of contaminant effects at the organismal level and below that have been utilized as biomarkers were pointed out. These include effects with some degree of chemical specificity and relationship to a mechanism of toxicity (AChE inhibition by organophosphate and carbamate insecticides, ALAD inhibition by lead, DNA–PAH adducts), responses associated with exposures to chemicals acting through a common receptor (Vtg induction by ER agonists such as natural and synthetic estrogens, some surfactants, plasticizers, and pesticides; CYP1A induction by AHR agonists such as certain pHAHs and PAHs), and broader indices of cellular stress or tissue damage, such as markers of oxidative stress, lysosomal membrane stability, and histopathology. Numerous other identified mechanisms of toxicity, indices of chemical exposure, and cellular and organismal impacts have been exploited, with varying degree of success, as biomarkers (see reviews by Huggett *et al.*, 1992; Peakall, 1992; Adams, 2002; van der Oost *et al.*, 2003). In addition, new biomarkers continue to emerge; for example, considerable attention is now being given to biomarkers arising from advances in genomic technologies, discussed above.

In considering the development or use of a selected biomarker, several issues and limitations warrant consideration. For example, while sensitivity is overall an advantage of many biomarkers, it can sometimes raise important questions surrounding interpretation. For example, some molecular and biochemical measures are very sensitive to chemical exposures, but their ramifications for organismal health are unclear. For this reason, some distinguish between biomarkers of exposure and biomarkers of effect (see reviews cited above). However, this distinction is often blurred and is subject to an individual's view of what constitutes a significant biological chemical effect; some may say the formation of DNA adduct is a significant effect, while others will argue that such adducts only indicate exposure and will require tumor formation to occur before denoting an effect. Certainly most would agree that the tumor is a clearer marker of effect than the adducts, and something more readily grasped by policy makers and the general public. On the other hand, tumors are far less sensitive as a biomarker, a key *raison d'etre*; such trade-offs merit consideration.

Chemical specificity among biomarkers is also highly variable and is imbued with trade-offs. In some cases, such as where one has a good idea of the nature of contaminants likely to occur at a site, chemical-specific biomarkers will likely be most informative. In contrast, if such information is lacking, or mixtures encompassing several classes of chemicals likely occur, nonspecific markers may be superior. In most cases, suites of biomarkers prove to be most effective, although the larger the suite, the more time intensive and costly the analysis will be, another trade-off. Another important consideration is the influence of the biomarker to variables other than those of concern (chemical contamination). Effects of environmental variables such as temperature, time of day or year, salinity and dissolved oxygen, and physiological variables such as sex, age, reproductive status, and nutritional status need to be controlled for or at least understood and accounted for. Many biomarkers are invasive and require sacrifice of the organism in order to obtain needed tissues. This can be problematic, particularly in cases involving rare species or charismatic species such as marine mammals. In such cases, and in others where feasible, the use of noninvasive biomarkers is either preferred or required (Fossi and Marsili, 1997). In summary, biomarkers can provide powerful tools as early warning signals of ecological damage, to assist in assessments of environmental contamination, and in determining the effectiveness of various environmental management decisions such as cleanups. However, careful case-specific thought must go into the selection of biomarkers, and they rarely are efficacious alone.

Population

Population-level effects are quantified with both field and laboratory approaches (see Newman, 1995, 2001). Population density is the most common of field population qualities measured in surveys of contaminated habitats. Quadrat, mark-recapture, and removal-based methods are applied. The density of individuals in a series of random quadrats within the area of interest is used to estimate densities in quadrat methods. The total population size can be estimated with knowledge of the total number of quadrats in the area of interest. In cases in which individuals are mobile and capable of avoiding being counted in a quadrat, a mark-recapture method might be applied instead. This involves marking a subset of individuals from the population, allowing them to randomly mix back into the population, and resampling the population. The number of marked and unmarked individuals taken, and the total number originally marked, can be used to estimate population size. Removal based methods involve repeated sampling of the population without replacement, noting how the number collected per unit of effort declines through the sequence of samplings, and extrapolating this trend down to the point (total number caught previous to a sampling) at which no more individuals will be taken. This point is an estimate of the population size. Obviously, this approach is useful only if sampling decreases the catch noticeably between sampling episodes.

As noted earlier in discussions of metapopulations, the spatial distribution of individuals in a habitat is important to understand. Fortunately, well established methods are available for this task. Methods vary depending on whether the sampling units are discrete or arbitrary. An arbitrary unit might be the number of razor clam per square meter of beach or number of a zooplankton species per cubic meter of water. A discrete sampling unit might be the number of mallard ducks per pond or squirrels per oak tree. Some methods associated with discrete sampling units attempt to fit the spatial pattern to a specific distribution. Methods for arbitrary sampling units include quadrat-based or distance-to-nearest-neighbor approaches as described by Krebs (1998).

Demographic surveys or experiments can be conducted for exposed populations. Some studies explore age-specific vital rates but others are designed to explore vital rates for different life ages such as nestling, fledgling, juvenile, and adult. Most result in data

sets that can be analyzed profitably using either a simple life table or more involved matrix analysis. The matrix method allows one to describe the population state and also to understand the sensitivity of the population to effects occurring to vital rates for various ages or stages (Caswell, 2001). The value of such studies lies in the ability to integrate effects to several effects into a projection of population consequences. Demographic studies are becoming more common in ecotoxicology, especially with species amenable to laboratory manipulation (Jansen *et al.*, 2001; Tanaka and Nakanishi, 2001; Chandler *et al.*, 2004).

AQ1

Conventional studies of increased tolerance after generations of exposure and molecular genetic surveys of exposed populations are the primary approaches by which genetic consequences are assessed. Increased tolerance is usually detected by subjecting individuals from the chronically exposed population and a naïve population to toxicant challenge and formally testing for tolerance differences. A recent example is the study by Ownby *et al.* (2002) of enhanced tolerance for a PAH-exposed population of killifish from Elizabeth River (Virginia). Alternatively, a change associated with a tolerance mechanism might be examined in chronically exposed and naïve populations. As an example, Meyer *et al.* (2003) found upregulated antioxidant defenses in the same populations of exposed Elizabeth River killifish studied by Ownby *et al.* (2002). Close examinations of population genetics associated with contaminated habitats are also used to infer consequences of multigenerational exposure. Continuing with the Elizabeth River killifish example, Mulvey *et al.* (2002, 2003) examined the genetic qualities of fish sampled within the Elizabeth River estuary using allozymes and mDNA. Clear evidence was found using both tools for the influence of contamination on the population genetics of killifish subpopulations within the estuary.

Community and Ecosystem

Most community and ecosystem effects studies by ecotoxicologists use modified methods developed in community and systems ecology (see Magurran, 1988, for method descriptions). Recent books such as Newman (1995) and Clements and Newman (2002) provide some details of ecotoxicological applications of these methods. Several general approaches are taken. The approach affording the most control and ability to replicate treatments involves laboratory microcosms. A microcosm is a simplified system that is thought to possess the community or ecosystem qualities of interest. The experimental control and reproducibility associated with microcosms come at the cost of losing ecological realism. Is the laboratory microcosm actually responding in a way that provides insight about how the actual community or ecosystem would respond? Microcosm studies are so common throughout the ecotoxicological literature that standard methods have been proposed for their execution (Taub, 1997). As a microcosm example, Clarke (1999) established invertebrate communities in the laboratory to determine the influence of oil drilling muds on offshore benthic communities. Relative to the issue of community redundancy discussed above, zinc-amended soil microcosms were used in another case by Salminen *et al.* (2001) and provided minimal evidence to support the current reliance on the redundant species theory by ecological risk assessors. Gaining back some realism by giving up some degree of tractability, outdoor mesocosms are also applied to community and ecosystem ecotoxicology. Mesocosms are larger experimental systems, usually constructed outdoors that also attempt to simulate some aspect of an ecosystem such as community species composition. Often, terrestrial ecotoxicologists apply the term enclosure instead of mesocosm for such experimental units. Aquatic mesocosms can be artificial ponds such as those developed by Woin (1998), streams such as those used by Kreutzweiser *et al.* (2000), or river segments such as those used by Culp *et al.* (2000). Terrestrial mesocosms can be pens, enclosures, or large soil plots depending on the effects being quantified. An example of a terrestrial mesocosm study is that conducted by Korthals *et al.* (1996) of the effects of long-term copper exposure to soil nematode communities. Field studies are the third means of exploring effects at the community or ecosystem level. The high realism of associated findings from field studies is balanced against the difficulty of achieving true replication and sufficient control of other factors influencing the system's response. Field studies can involve manipulations such as introducing toxicant into replicate water bodies; however, the majority of field studies involve biomonitoring of an existing, notionally impacted, community or ecosystem. This might involve close examination of species composition and comparison to that expected or measured in a similar, but uncontaminated, system. Most biomonitoring efforts focus on community structure instead of function because it is generally believed that changes in community structure will be seen before those in functions. As examples, metal effects on invertebrate and plant community structure were studied by Peeters *et al.* (2000) and Strandberg *et al.* (2006), respectively. Despite the tendency to study community structure, study of functions can provide valuable insights as in the case of Day (1993), who found changes in photosynthesis in periphytic algae in response to herbicide exposure. Because mesocosm and field studies involve data generation in the presence of many uncontrolled variables and poor replication or pseudoreplication, multivariate statistical techniques for recognizing patterns among locations or through time are commonly applied, for example, Landis *et al.* (1997) and Kedwards *et al.* (1999).

Landscape to Biosphere

The creation and eventual convergence of several key technologies facilitate ecotoxicological study at the landscape to biosphere vantages. These same technologies have also allowed the emergence of large context, environmental disciplines such as landscape[4] ecology (Forman and Godron, 1986), global ecology (Rambler *et al.*, 1989), and global biogeochemistry (Butcher *et al.*, 1992) that contribute concepts to large-scale ecotoxicology efforts.

Technologies for acquiring, processing, and analyzing large amounts of information have been essential. Archived and new imagery from satellites and high-altitude platforms is now integrated with off-the-shelf GIS software with affordable computers. Much of this imagery is gathered with remote sensing technologies, that is, technologies that do not require physical contact with the feature being measured. However, arrays of sensors are rapidly coming together such as the network coastal observing systems that are quickly linking to form a readily accessible real time data stream for all of our oceans. Remote sensing data from satellites or aircraft provide information for wide spatial areas and the rapidly emerging, ground- or water-based observing system networks have begun to produce extremely rich data streams. Such technologies facilitate ecotoxicological explorations at spatial scales that were impossible to consider only a few decades ago.

[4]A landscape is formally defined by Forman and Godron (1986) as a heterogeneous landscape that is "composed of a cluster of interacting ecosystems that is repeated in a similar form throughout." Each ecosystem is a part of a whole landscape much as a tessera is part of a mosaic or a subpopulation is part of a metapopulation.

ECOLOGICAL RISK ASSESSMENT

ERA applies ecotoxicological knowledge in support of environmental decision making. The ERA approach is an adaptation of human risk assessment methods, notably those articulated in the National Academy of Sciences paradigm (National Research Council, 1983). Adaptations are needed to accommodate differences in exposure pathways and the entities for which risk is to be estimated. Risk might be to an endangered or threatened species, or to a damaged natural resource for which remuneration might be required from a responsible party. In such cases, the ERA might estimate risk to individuals. Alternatively, as emphasized in the above EPA quote, the risk might be to a local species population or to the integrity of an ecological community. A widely dispersed ecotoxicant such as acid precipitation or widely used product such as the herbicide, atrazine, might require assessment of risk at a landscape or subcontinental scale. A recent example of such a risk assessment is that for atrazine, a herbicide used throughout North America (Solomon *et al.*, 1996). Ecotoxicants requiring a global ERA might include greenhouse gases contributing to global warming, hydrofluorocarbons depleting the ozone layer, and POPs that accumulate to harmful concentrations in polar regions far from their point of release at highly industrialized latitudes.

Adaptations are based on the context of an ERA. Some ERAs address existing situations. Considerable field information might be available for such a retroactive ERA and epidemiological methods might be applied advantageously. In contrast, predictive ERAs assess possible risk associated with a future or proposed toxicant exposure. In this case, the ERA might rely more heavily on exposure modeling and laboratory derived effects data. A special case of predictive risk assessment is a life cycle assessment in which "cradle-to-grave" predictions are done for a product that includes all aspects of its raw material extraction, manufacture, distribution, use, and final disposal. Finally, an ERA will be structured slightly differently if it compares the ecological risk of one or more options. An example would be the comparative risk associated with a spill of bunker oil versus Orimulsion® (a bitumen-based fossil fuel). Such a comparative risk assessment might draw insight and data from existing spill sites, laboratory tests, and exposure models. Despite adaptations and differing contexts, most ERAs have the same general form (see Fig. 30-2).

Risk assessors, risk managers, and key stakeholders engage in initial planning together with the intention of formulating a clear statement of the problem. What valued ecological entity or quality is being assessed (assessment end point) is defined. A conceptual model is created that links the assessment end point and the toxicant, including descriptions of exposure pathways and possible effects. A clear statement of possible or predicted effects (risk hypothesis) is formulated. A clear formulation of the problem with concurrence of key stakeholders is critical to the ERA because of the diversity of possible assessment end points and exposure pathways.

Exposure characterization describes or predicts contact between the toxicant and the assessment end point. Depending on the ERA context, this could involve a simple calculation of average exposure, or a temporally and spatially explicit description of amounts present in relevant media. Toxicant sources, transport pathways, kinds of contact, and potential costressors are also defined.

Ecological effects characterization describes the qualities of any potential effects of concern, describes the connection between the potential effects and the assessment end point, and describes how changes in the level of exposure might influence the effects manifesting in the assessment end point. Normally, a statement about the strength of evidence associated with the descriptions is presented in the ecological effects characterization. As a common example of evidentiary uncertainty often requiring explanation is the measurement end point. It is not always desirable to derive effects information directly from an assessment end point such as an endangered species so uncertainty is introduced by gauging effects to a surrogate (measurement end point). Ecological effects characterizations must describe the justifiable confidence in extrapolating from measurement to assessment end points.

Risk characterization uses the analysis of exposure and ecological effects to address the risk question(s) posed in the problem formulation. This can involve an explicit statement of risk, that is, the probability of a specified intensity of an adverse effect occurring to the assessment end point. Often, the information needed to make such an explicit statement is absent and a qualitative statement of the likelihood of an adverse effect is made instead. Regardless of whether a quantitative or qualitative statement of risk is produced, the risk characterization must provide details surrounding the statement, including important uncertainties.

INTERCONNECTIONS BETWEEN ECOSYSTEM INTEGRITY AND HUMAN HEALTH

As noted at the beginning of this chapter, while the original definition of ecotoxicology included effects on humans, most subsequent treatments exclude discussions of humans except as a source of contaminants, with some notable exceptions (eg, Newman and Unger, 2003). This ecotoxicology chapter, imbedded in a book focused on human or biomedical toxicology, describes the younger science of elucidating chemical effects in natural systems. While ecotoxicology has features distinct from biomedical toxicology, it is important to consider parallelisms in the two fields and, more broadly, interconnections between human health and ecological integrity, or health. While obviously related, biomedical and ecological toxicology have historically exhibited relatively little coordination or collaboration among scientists across these fields. This is likely due to a number of reasons, including the different levels of biological organization considered, as well as different academic cultures populating the two fields. However, it is questionable if this gulf has been in the best interest of understanding chemical effects, and ultimately protecting both human and ecological health. This concern has prompted several broad discussions intended to bridge this divide and enhance interdisciplinary natural and social scientific research in these areas (see reviews by Costanza *et al.*, 1992; Di Giulio and Monosson, 1996; Di Giulio and Benson, 2002).

This gulf has resulted in two fields that, while largely disconnected, parallel one another and share common paradigms such as dose–response, toxicokinetics, mechanisms of action, and risk assessment frameworks. However, by generally ignoring how chemicals and other anthropogenic stressors that degrade ecosystems can ultimately impact human health and well-being, and vice versa, an opportunity to holistically understand the results of environmental contamination is lost. Miranda *et al.* (2002) developed a conceptual model for elucidating the interconnections paradigm that links natural and social systems in a circular manner with continuous feedbacks, as opposed to parallel linear models (exposure to response, in either humans or ecosystems) that dominate toxicology currently. In this conceptual model, the natural system produces both positive outputs (such as natural resources, raw materials) and negative outputs (eg, hurricanes, disease vectors) to the social system. The culture and institution of the social system in turn transforms the natural system outputs in various ways and subsequently delivers

various positive outputs (consumer goods, conservation efforts) and negative outputs (pollution, deforestation) to the natural system. These outputs influence the quantity and quality of life (human and nonhuman) of the natural system, and the circular flow of resources continually creates conditions that influence the well-being of individuals, societies, and ecosystems, now and in the future.

This rather abstract model formalizes the interconnections between human and ecological health that most of us intuitively sense. Some of these connections, in the context of environmental pollution, are obvious. Chemical contamination of seafoods valued by humans is one example. Others are less clear but potentially very significant, such as human impacts on aquatic systems that foster the propagation of human disease vectors, or human impacts on global climate that may concomitantly impact humans and ecosystems in varied and complex ways. Also important to consider is the matter of human perceptions of their environment; people's sense of the health of the environment in which they live (whether their perception is correct or not) can have substantial impacts on their mental and physical health (O'Keefe and Baum, 1996). As noted by Kendall *et al.* (2001), "the indirect effects of environmental pollution may, in the end, be more important than the direct effects for human health."

There are significant indications that these fields are converging and meaningful interactions are increasing. The inclusion of an ecotoxicology chapter in this text is favorable evidence. This trend is motivated in part by the genomics revolution that provides powerful methods for evaluating fundamental biological similarities across species, including those employed in biomedical and ecotoxicological research. Research in this area has revealed genetic similarities, or conservation, in many genes and the proteins they code for that are important to organismal adaptations and impacts due to environmental stressors, including chemicals (Eaton *et al.*, 2006). Certainly many important species differences also exist that contribute to the great complexity of understanding human ecological interconnections, but as pointed out by Winston *et al.* (2002), "in the final analysis, the biological similarities across living systems are probably more impressive than the differences." Discussions among diverse scientists of the promises, limitations, and potential applications of genomics for elucidating cross-species extrapolations are provided by Benson and Di Giulio (2006). Such cross-fertilizations among biomedical and environmental scientists, as well as social scientists and policy makers, are likely to enhance all areas, and catalyze the integrated protection of human and ecosystem health.

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REFERENCES

Adams SM. Evaluating effects of contaminants on fish health at multiple levels of biological organization: extrapolating from lower to higher levels. *Hum Ecol Risk Assess.* 2000;6:15–27.

Adams SM, Shepard KL, Greeley MS, Jimenez BD, Ryon MG, Shugart LR. The use of bioindicators for assessing the effects of pollutant stress on fish. *Mar Environ Res.* 1989;28:459–464.

Allison N, Millward GE, Jones MB. Particle processing by *Mytilus edulis*: effects on bioavailability of metals. *J Exp Mar Biol Ecol.* 1998;22:149.

Alfred PM, Gilesy JP. Use of in situ microcosms to study mass loss and chemical composition of leaf litter being processed in a blackwater stream. *Arch Hydrobiol.* 1988;114:231.

Amat A, Pfol-Leskowicz A, Burgeot T, Castegnaro M. DNA adducts as a biomarker of pollution: field study on the genotoxic impact evolution of the *Erika* oil spills on mussels (*Mytilus edulis*) over a period of eleven months. *Polycyclic Aromatic Compounds.* 2004;24:713–732.

Anders A. *Biostatistics for Epidemiologists.* Boca Raton, FL: CRC Press; 1993.

Anderson DW, Jehl JR, Risebrough RW, Woods LA, Deweese LR, Edgecomb WG. Brown pelicans: improved reproduction off the Southern California Coast. *Science.* 1975;190:806.

Ankley GT, Daston GP, Degitz SJ, et al. Toxicogenomics in regulatory ecotoxicology. *Environ Sci Technol.* 2006;40:4055–4065.

Ankley GT, Erickson RJ, Sheedy BR, et al. Evaluation of models for predicting the phototoxic potency of polycyclic aromatic hydrocarbons. *Aquat Toxicol.* 1997;37:37–50.

Antkiewicz DS, Burns CG, Carney SA, Peterson RE, Heideman W. Heart malformation is an early response to TCDD in embryonic zebrafish. *Toxicol Sci.* 2005;84:368–377.

Arfsten DP, Schaeffer DJ, Mulveny DC. The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: a review. *Ecotoxicol Environ Saf.* 1996;33:1–24.

ASTM International. *Annual Book of ASTM Standards, Volume 11.05, Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Responses.* West Conshohocken, PA: ASTM International; 2006.

ATSDR. *Toxicological Profile for Lead.* Washington, DC: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; 1999.

Aubone A. Loss of stability owing to a stable age structure skewed toward juveniles. *Ecol Model.* 2004;175:55.

Auffret M, Rousseau S, Boutet I, et al. A multiparametric approach for monitoring immunotoxic responses in mussels from contaminated sites in Western Mediterranean. *Ecotoxicol Environ Saf.* 2006;63:393–405.

Bainy ACD, Saito E, Carvalho PSM, Junqueira VBC. Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia (*Oreochromis niloticus*) from a polluted site. *Aquat Toxicol.* 1996;34:151–162.

Baker CF, Montgomery JC. Sensory deficits induced by cadmium in banded kokopu, *Galaxias fasciatus*, juveniles. *Environ Biol Fishes.* 2001;62:455–464.

Baldwin DH, Sandahl JF, Labenia JS, Scholz NL. Sublethal effects of copper on coho salmon: impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environ Toxicol Chem.* 2003;22:2266–2274.

Basu N, Klenavic K, Gamberg M, et al. Effects of mercury on neurochemical receptor-binding characteristics in wild mink. *Environ Toxicol Chem.* 2005;24:1444–1450.

Bauler MB, Palace VP, Hodson PV. Is oxidative stress the mechanism of blue sac disease in retene-exposed trout larvae? *Environ Toxicol Chem.* 2005;24:694–702.

Baumann PC. Epizootics of cancer in fish associated with genotoxins in sediment and water. *Mutat Res Rev Mutat Res.* 1998;411:227–233.

Baumann PC, Harshbarger JC. Long term trends in liver neoplasm epizootics of brown bullhead in the Black River, Ohio. *Environ Monit Assess.* 1998;53:213–223.

Benson WH, Di Giulio RT, eds. *Emerging Molecular and Computational Approaches for Cross-Species Extrapolations.* New York: Taylor & Francis; 2006.

Biesmeijer JC, Roberts SPM, Reemer M, et al. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science.* 2006;313:351.

Billiard SM, Querbach K, Hodson PV. Toxicity of retene to early life stages of two freshwater fish species. *Environ Toxicol Chem.* 1999;18:2070–2077.

Billiard SM, Timme-Laragy AR, Wassenberg DM, Cockman C, Di Giulio RT. The role of the aryl hydrocarbon receptor pathway in mediating synergistic developmental toxicity of polycyclic aromatic hydrocarbons to zebrafish. *Toxicol Sci.* 2006;92:526–536.

Birdsall CW, Grue CE, Anderson A. Lead concentrations in bullfrog (*Rana catesbeiana*) and green frog (*Rana clamitans*) tadpoles inhabiting highway drainages. *Environ Pollut.* 1986;40A:233–247.

Broman D, Naf C, Rolff C, Zebuhr Y, Fry B, Hobbie J. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the Northern Baltic. *Environ Toxicol Chem.* 1992;11:331.

AQ5

Broomhall S. The effects of endosulfan and variable water temperature on survivorship and subsequent vulnerability to predation in *Litoria citropa* tadpoles. *Aquat Toxicol.* 2002;61:243.

Brown RE, Brain JD, Wang N. The avian respiratory system: a unique model for studies of respiratory toxicosis and for monitoring air quality. *Environ Health Perspect.* 1997;105:188–200.

Bury NR. The changes to apical silver membrane uptake, and basolateral membrane silver export in the gills of rainbow trout (*Oncorhynchus mykiss*) on exposure to sublethal silver concentrations. *Aquat Toxicol.* 2005;72:135–145.

Bustnes JO, Miland O, Fjeld M, et al. Relationships between ecological variables and four organochlorine pollutants in an artic glaucous gull (*Larus hyperboreus*) population. *Environ Pollut.* 2005;136:175–185.

Butcher SS, Charlson RJ, Orians GH, Wolfe GV. *Global Biogeochemical Cycles.* London: Academic Press; 1992.

Butler RA, Kelley ML, Powell WH, Hahn ME, Van Beneden RJ. An aryl hydrocarbon receptor (AHR) homologue from the soft-shell clam, *Mya arenaria*: evidence that invertebrate AHR homologues lack 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and beta-naphthoflavone binding. *Gene.* 2001;278:223–234.

Campbell PGC, Tessier A. Ecotoxicology of metals in the aquatic environment: geochemical aspects. In: Newman MC, Jagoe CH, eds. *Ecotoxicology. A Hierarchical Treatment.* Boca Raton, FL: CRC Press; 1996:11.

Carls MG, Rice SD, Hose JE. Sensitivity of fish embryos to weathered crude oil: part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environ Toxicol Chem.* 1999;18:481–493.

Carney SA, Peterson RE, Heideman W. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin activation of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator pathway causes developmental toxicity through a CYP1A independent mechanism in zebrafish. *Mol Pharmacol.* 2004;66:512–521.

Carney SA, Prasch AL, Heideman W, Peterson RE. Understanding dioxin developmental toxicity using the zebrafish model. *Birth Defects Res A.* 2006;76:7–18.

Carr RL, Ho LL, Chambers JE. Selective toxicity of chlorpyrifos to several species of fish during an environmental exposure: biochemical mechanisms. *Environ Toxicol Chem.* 1997;16:2369–2374.

Carson R. *Silent Spring.* Boston: Houghton Mifflin; 1962.

Carvalho PSM, Tillitt DE. 2,3,7,8-TCDD effects on visual structure and function in swim-up rainbow trout. *Environ Sci Technol.* 2004;38:6300–6306.

Caswell H. *Matrix Population Models. Construction, Analysis, and Interpretation.* Sunderland, MA: Sinauer Associates; 2001.

Chandler GT, Cary TL, Bejarano AC, Pender J, Ferry JL. Population consequences of fipronil and degradates to copepods at field concentrations: an integration of life cycle testing with Leslie matrix population modeling. *Environ Sci Technol.* 2004;38:6407.

Chapman PM. Indirect effects of contaminants. *Mar Pollut Bull.* 2004; 48:411.

Chaty S, Rodius F, Vasseur P. A comparative study of the expression of CYP1A and CYP4 genes in aquatic invertebrate (freshwater mussel, *Unio tumidus*) and vertebrate (rainbow trout, *Oncorhynchus mykiss*). *Aquat Toxicol.* 2004;69:81–93.

Chen Z, Mayer LM. Mechanisms of Cu solubilization during deposit feeding. *Environ Sci Technol.* 1998;32:770.

Cherkasov AS, Biswas PK, Ridings DM, Ringwood AH, Sokolova IM. Effects of acclimation temperature and cadmium exposure on cellular energy budgets in the marine mollusk *Crassostrea virginica*: linking cellular and mitochondrial responses. *J Exp Biol.* 2006;209:1274–1284.

Chernyak SM, McConnell LL, Rice CP. Fate of some chlorinated hydrocarbons in arctic and far eastern ecosystems in the Russian Federation. *Sci Total Environ.* 1995;75:160–161.

Chiou CT. Partition coefficients of organic compounds in lipid–water systems and correlations with fish bioconcentration factor. *Environ Sci Technol.* 1985;19:57.

Chiverton PA. The benefits of unsprayed cereal crop margins to grey partridge *Perdix perdix* and pheasants *Phasianus colchicus* in Sweden. *Wildl Biol.* 1999;5:8.

Chowdhury MJ, Pane EF, Wood CM. Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout: respiratory, ionoregulatory, and stress parameters. *Comp Biochem Physiol.* 2004;139C:163–173.

Clark AJ, Scheuhammer AM. Lead poisoning in upland-foraging birds of prey in Canada. *Ecotoxicology.* 2003;12:23–30.

Collier TK, Singh SV, Awasthi YC, Varanasi U. Hepatic xenobiotic metabolizing enzymes in 2 species of benthic fish showing different prevalences of contaminant-associated liver neoplasms. *Toxicol Appl Pharmacol.* 1992;113:319–324.

Connell DW. *Bioaccumulation of Xenobiotic Compounds.* Boca Raton, FL: CRC Press; 1990.

Cook PM, Robbins JA, Endicott DD, et al. Effects of aryl hydrocarbon receptor-mediated early life stage toxicity on lake trout populations in Lake Ontario during the 20th century. *Environ Sci Technol.* 2003;37: 3864–3877.

Costanza R, Norton BG, Haskell BD, eds. *Ecosystem Health. New Goals for Environmental Management.* Washington, DC: Island Press; 1992.

Crollius HR, Weissenbach J. Fish genomics and biology. *Genome Res.* 2005;15:1675–1682.

Culp JM, Lowell RB, Cash KJ. Integrating mesocosm experiments with field and laboratory studies to generate weight-of-evidence risk assessments for large rivers. *Environ Toxicol Chem.* 2000;19:1167.

Custer TW, Custer CM, Dickerson K, Allen K, Melancon MJ, Schmidt LJ. Polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, trace elements, and monooxygenase activity in birds nesting on the North Platte River, Casper, Wyoming, USA. *Environ Toxicol Chem.* 2001;20:624–631.

Custer TW, Custer CM, Hines RK, et al. Organochlorine contaminants and reproductive success of double-crested cormorants from Green Bay, Wisconsin, USA. *Environ Toxicol Chem.* 1999;18:1209–1217.

Day KE. Short-term effects of herbicides on primary productivity of periphyton in lotic environments. *Ecotoxicology.* 1993;2:123.

de Andrade VM, da Silva J, Heuser VD, Dias JF, Yoneama ML, de Freitas TRO. Fish as bioindicators to assess the effects of pollution in two Southern Brazilian rivers using the comet assay and micronucleus test. *Environ Mol Mutagen.* 2004;44:459–468.

Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol.* 2003;43:309–334.

Deroo BJ, Korach KS. Estrogen receptors and human disease. *J Clin Invest.* 2006;116:561–570.

Devlin EW. Acute toxicity, uptake and histopathology of aqueous methyl mercury to fathead minnow embryos. *Ecotoxicology.* 2006;15:97–110.

Di Giulio RT, Hinton DE, eds. *The Toxicology of Fishes.* CRC Press. In press. AQ6

Di Giulio RT, Monosson E, eds. *Interconnections between Human and Ecosystem Health.* London: Chapman and Hall; 1996.

Di Giulio RT, Washburn PC, Wenning RJ, Winston GW, Jewell CS. Biochemical responses in aquatic animals: a review of determinants of oxidative stress. *Environ Toxicol Chem.* 1989;8:1103–1123.

Di Toro DM, Mahony JD, Hansen DJ, et al. Toxicity of cadmium in sediments: the role of acid volatile sulfide. *Environ Toxicol Chem.* 1990;9:1487.

Di Toro DM, McGrath JA, Hansen DJ, et al. Predicting sediment metal toxicity using a sediment biotic ligand model: methodology and initial application. *Environ Toxicol Chem.* 2005;24:2410–2427.

Dopp E, Barker CM, Schiffmann D, Reinisch CL. Detection of micronuclei in hemocytes of *Mya arenaria*: association with leukemia and induction with an alkylating agent. *Aquat Toxicol.* 1996;34:31–45.

Dorval J, Leblond V, Deblois C, Hontela A. Oxidative stress and endocrine endpoints in white sucker (*Catostomus commersoni*) from a river impacted by agricultural chemicals. *Environ Toxicol Chem.* 2005;24:1273–1280.

du Corbier FA, Lyons BP, Stentiford GA, Rotchell JM. The role of the retinoblastoma gene in liver tumor development in fish. *Environ Sci Technol.* 2005;39:9785–9790.

Eaton D, Gallagher E, Hooper M, et al. Species differences in response to toxic substances: shared pathways of toxicity—values and limitations of omic technologies to elucidate mechanism or mode of action. In: Benson WH, Di Giulio RT, eds. *Emerging Molecular and Computational Approaches for Cross-Species Extrapolations.* New York: Taylor & Francis; 2006.

Environmental Protection Agency. *Summary Report on Issues in Ecological Risk Assessment.* EPA/625/3-91/018. Springfield, VA: NTIS; 1991.

Evans DH. The fish gill: site of action and model for toxic effects of environmental pollutants. *Environ Health Perspect.* 1987;71:47–58.

Evans MI, Syemens P, Pilcher CWT. Short-term damage to coastal bird populations in Saudi-Arabia and Kuwait following the 1991 Gulf War marine pollution. *Mar Pollut Bull.* 1993;27:157–161.

Fairbrother A, Ankley GT, Birnbaum LS, et al. Reproductive and developmental toxicology of contaminants in oviparous animals. In: Di Giulio RT, Tillitt DE, eds. *Reproductive and Developmental Effects of Contaminants in Oviparous Vertebrates.* Fairmont, MT: SETAC Press; 1999:283–361.

Fairbrother A, Smits J, Grasman KA. Avian immunotoxicology. *J Toxicol Environ Health Crit Rev.* 2004;7B:105–137.

Fernandez P, Grimait JO. On the global distribution of persistent organic pollutants. *CHIMIA.* 2003;57:514.

Fernie KJ, Mayne G, Shutt JL, et al. Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. *Environ Pollut.* 2005;138:485–493.

Fleeger JW, Carman KR, Nisbet RM. Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ.* 2003;317:207.

Forbes VE, Calow P. Is per capita rate of increase a good measure of population-level effects in ecotoxicology? *Environ Toxicol Chem.* 1999;18:1544.

Forman RTT, Godron M. *Landscape Ecology.* New York: Wiley; 1986.

Fossi MC, Marsili L. The use of non-destructive biomarkers in the study of marine mammals. *Biomarkers.* 1997;2:205–216.

Fox G. Practical causal inference for ecoepidemiologists. *J Toxicol Environ Health.* 1991;33:359.

Fridovich I. Mitochondria: are they the seat of senescence? *Aging Cell.* 2004;3:13–16.

Fry DM. Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environ Health Perspect.* 1995;103(suppl 7):165–171.

Gard TC. Stochastic models for toxicant-stressed populations. *Bull Math Biol.* 1992;54:827.

Gardner SC, Oberdorster E. *Toxicology of Reptiles.* New York: Taylor and Francis; 2005.

Gilbertson M, Kubiak T, Ludwig J, et al. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds—similarity to chick edema disease. *J Toxicol Environ Health.* 1991;33:455–520.

Grue CE, Gilbert PL, Seeley ME. Neurophysiological and behavioral changes in non-target wildlife exposed to organophosphate and carbamate pesticides: thermoregulation, food consumption, and reproduction. *Am Zool.* 1997;37:369–388.

Grue CE, Powell GVN, McChesney MJ. Care of nestlings by wild female starlings exposed to an organo-phosphate pesticide. *J Appl Ecol.* 1982;19:327–335.

Guillette LJ, Crain DA, Gunderson MP, et al. Alligators and endocrine disrupting contaminants: a current perspective. *Am Zool.* 2000;40:438–452.

Hahn ME. Aryl hydrocarbon receptors: diversity and evolution. *Chem Biol Interact.* 2002;141:131–160.

Hahn ME, Merson RR, Karchner SI. Xenobiotic receptors in fish: structural and functional diversity and evolutionary insights. In: Mommsen TP, Moon TW, eds. *Environmental Toxicology, Vol 6, Biochemistry and Molecular Biology of Fishes.* Amsterdam: Elsevier; 2005.

Hall AJ, Hugunin K, Deaville R, et al. The risk of infection from polychlorinated biphenyl exposure in the *harbor porpoise* (*Phocoena phocoena*): a case-control approach. *Environ Health Perspect.* 2006;114:704–711.

Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine.* 3rd ed. Oxford, UK: Oxford University Press; 1999.

Handy RD, Runnalls T, Russell PM. Histopathologic biomarkers in three spined sticklebacks, *Gasterosteus aculeatus*, from several rivers in Southern England that meet the freshwater fisheries directive. *Ecotoxicology.* 2002;11:467–479.

Hankinson O. The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol.* 1995;35:307–340.

Harries JE, Sheahan DA, Jobling S, et al. Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environ Toxicol Chem.* 1997;16:534–543.

Harshbarger JC, Clark JB. Epizootiology of neoplasms of bony fish of North America. *Sci Total Environ.* 1990;94:1–32.

Hasspieler BM, Behar JV, Di Giulio RT. Glutathione-dependent defense in channel catfish (*Ictalurus punctatus*) and brown bullhead (*Ameiurus nebulosus*). *Ecotoxicol Environ Saf.* 1994;28:82–90.

Heath JA, Frederick PC. Relationships among mercury concentrations, hormones, and nesting effort of White Ibises (*Eudocimus albus*) in the Florida Everglades. *Auk.* 2005;122:255–267.

Heid SE, Walker MK, Swanson HI. Correlation of cardiotoxicity mediated by halogenated aromatic hydrocarbons to aryl hydrocarbon receptor activation. *Toxicol Sci.* 2001;61:187–196.

Henny CJ. Effects of mining lead on birds: a case history at Coeur d'Alene Basin, Idaho. In: Hoffman DJ, Rattner BA, Burton GA Jr, Cairns J Jr, eds. *Handbook of Ecotoxicology.* 2nd ed. Boca Raton, FL: Lewis; 2003.

Hill AB. The environment and disease: association or causation? *Proc R Soc Med.* 1965;59:295.

Hinton DE. Cells, cellular responses, and their markers in chronic toxicity. In: Malins DC, Ostrander GK, eds. *Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspectives.* Boca Raton, FL: Lewis; 1994:207–239.

Hippeli S, Elstner EF. Mechanisms of oxygen activation during plant stress: biochemical effects of air pollutants. *J Plant Physiol.* 1996;148:249–257.

Hoffman DJ, Marn CM, Marois KC. Sublethal effects in avocet and stilt hatchlings from selenium contaminated sites. *Environ Toxicol Chem.* 2002;21:561–566.

Hoffman DJ, Rattner BA, Burton GA, Cairns J, eds. *Handbook of Ecotoxicology.* Boca Raton, FL: Lewis/CRC Publishers; 2003.

Holm J, Palace V, Siwik P, et al. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. *Environ Toxicol Chem.* 2005;24:2373–2381.

Hontela A. Endocrine and physiological responses to xenobiotics in fish: role of glucocorticoid hormones. *Rev Toxicol.* 1997;1:1–46.

Horness BH, Lomax DP, Johnson LL, Myers MS, Pierce SM, Collier TK. Sediment quality thresholds: estimates from hockey stick regression of liver prevalence in English sole (*Pleuronectes vetulus*). *Environ Toxicol Chem.* 1998;17:872.

Hose JE, Cross JN, Smith SG, Diehl DL. Elevated circulating erythrocyte micronuclei in fishes from contaminated sites off southern California. *Mar Environ Res.* 1987;22:167–176.

Huang YW, Matthews JB, Fertuck KC, Zacharewski TR. Use of *Xenopus laevis* as a model for investigating in vitro and in vivo endocrine disruption in amphibians. *Environ Toxicol Chem.* 2005;24:2002–2009.

Huggett RJ, Kimerle RA, Mehrle PM Jr, Bergman HL, eds. *Biomarkers. Biochemical, Physiological, and Histological Markers of Anthropogenic Stress.* Boca Raton, FL: Lewis; 1992.

Husby MP, McBee K. Nuclear DNA content variation and double-strand DNA breakage in white-footed mice (*Peromyscus leucopus*) collected from abandoned strip mines, Oklahoma, USA. *Environ Toxicol Chem.* 1999;18:926–931.

Hwang HM, Wade TL, Sericano JL. Relationship between lysosomal membrane destabilization and chemical body burden in eastern oysters (*Crassostrea virginica*) from Galveston Bay, Texas, USA. *Environ Toxicol Chem.* 2002;21:1268–1271.

Incardona JP, Carls MG, Teraoka H, et al. Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. *Environ Health Perspect.* 2005;113:1755–1762.

Jansen A, Forbes VE, Parker ED Jr. Variation in cadmium uptake, feeding rate, and life history effects in the gastropod *Potamopyrgus antipodarum*: linking toxicant effects on individuals to the population level. *Environ Toxicol Chem.* 2001;20:2503.

Johnson L, Casillas E, Sol S, Collier T, Stein J, Varanasi U. Contaminant effects on reproductive success in selected benthic fish. *Mar Environ Res.* 1993;35:165–170.

Johnson LL, Stein JE, Collier TK, Casillas E, Varanasi U. Indicators of reproductive development in prespawning female winter flounder

(*Pleuronectes americanus*) from urban and nonurban estuaries in the northeast United States. *Sci Total Environ.* 1994;141:241–260.

Kammenga J, Laskowski R. *Demography in Ecotoxicology*. Chichester, UK: Wiley; 2000.

Kappus H. Overview of enzyme systems involved in bio-reduction of drugs and in redox cycling. *Biochem Pharmacol.* 1986;35:1–6.

Karr JR. Biological integrity: a long-neglected aspect of water resource management. *Ecol Appl.* 1991;1:66.

Kedwards TJ, Maund SJ, Chapman PF. Community level analysis of ecotoxicological field studies: I. Biological monitoring. *Environ Toxicol Chem.* 1999;18:149.

Keller JM, McClellan-Green PD, Kucklick JR, Keil DE, Peden-Adams MM. Effects of organochlorine contaminants on loggerhead sea turtle immunity: comparison of a correlative field study and in vitro exposure experiments. *Environ Health Perspect.* 2006;114:70–76.

Kendall RJ, Anderson TA, Baker RJ, et al. Ecotoxicology. In: Klaassen CD, ed. *Casarett and Doull's Toxicology, the Basic Science of Poisons*. 6th ed. New York: McGraw-Hill; 2001.

Kendall RJ, Lacher TE Jr, Bunck C, et al. An ecological risk assessment of lead shot exposure in non-waterfowl avian species: upland game birds and raptors. *Environ Toxicol Chem.* 1996;15:4–20.

Kidd KA, Bootsma HA, Hesslein RH, Muir DCG, Hecky RE. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. *Environ Sci Technol.* 2001;35:14–20.

Kohler A, Wahl E, Soffker K. Functional and morphological changes of lysosomes as prognostic biomarkers of toxic liver injury in a marine flatfish (*Platichthys flesus* (L.)). *Environ Toxicol Chem.* 2002;21:2434–2444.

Korthals GW, Alexiev AD, Lexmond TM, Kammenga JE, Bongers T. Long-term effects of copper and pH on the nematode community in an agroecosystem. *Environ Toxicol Chem.* 1996;15:979.

Krebs CJ. *Ecological Methodology*. 2nd ed. New York: Benjamin Cummings; 1998.

Kreutzweiser DP, Capell SS, Scarr TA. Community-level responses by stream insects to NEEM products containing azadirachtin. *Environ Toxicol Chem.* 2000;19:855.

Krivtsov V. Investigations of indirect relationships in ecology and environmental science: a review and the implications for comparative theoretical ecosystem analysis. *Ecol Model.* 2004;174:37.

Landis WG, Matthews RA, Matthews GB. Design and analysis of multispecies toxicity tests for pesticide registration. *Ecol Appl.* 1997;7:1111.

Larson RA, Weber EJ. *Reaction Mechanisms in Environmental Organic Chemistry*. Boca Raton, FL: Lewis Publishers; 1994.

Larsson DGJ, Adolfsson-Erici M, Parkkonen J, et al. Ethinyloestradiol—an undesired fish contraceptive? *Aquat Toxicol.* 1999;45:91–97.

Lee B-G, Griscom SB, Lee J-K, et al. Influences of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments. *Science.* 2000;287:282.

Lemly AD. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. *Aquat Toxicol.* 2002;57:39–49.

Leslie HA, Oosthoek AJP, Busser FJM, Kraak MHS, Hermens JLM. Biomimetic solid-phase microextraction to predict body residues and toxicity of chemicals that act by narcosis. *Environ Toxicol Chem.* 2002;21:229.

Li J, Quabius ES, Wendelaar Bonga SE, Flik G, Lock RAC. Effects of water-borne copper on branchial chloride cells and Na^+/K^+-ATPase activities in Mozambique tilapia (*Oreochromis mossambicus*). *Aquat Toxicol.* 1998;43:1–11.

Linbo TL, Stehr CM, Incardona JP, Scholz NL. Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. *Environ Toxicol Chem.* 2006;25:597–603.

Lipscomb TP, Harris RK, Rebar AH, Ballachey BE, Haebler RJ. *Exxon Valdez Oil Spill State/Federal Natural Resource Damage Assessment, Final Report. Pathological Studies of Sea Otters (Marine Mammal Study 6–11)*. Anchorage, AK: US FWS; June 1996.

Little PFR. Structure and function of the human genome. *Genome Res.* 2005;15:1759–1766.

Livingstone DR. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar Pollut Bull.* 2001;42:656–666.

Lloyd R, Herbert DWM. The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). *Ann Appl Biol.* 1960;48:399.

Loge FJ, Arkoosh MR, Ginn TR, Johnson LL, Collier TK. Impact of environmental stressors on the dynamics of disease transmission. *Environ Sci Technol.* 2005;39:7329–7336.

Loreau M. Does functional redundancy exist? *Oikos.* 2004;104:606.

Lorenzen A, Williams KL, Moon TW. Determination of the estrogenic and antiestrogenic effects of environmental contaminants in chicken embryo hepatocyte cultures by quantitative-polymerase chain reaction. *Environ Toxicol Chem.* 2003;22:2329–2336.

Luoma SN. Can we determine the biological availability of sediment-bound trace elements? *Hydrobiologia.* 1989;379:176–177.

Luoma SN, Bryan GW. Factors controlling the availability of sediment-bound lead to the estuarine bivalve *Scrobicularia plana*. *J Mar Biol Assoc UK.* 1978;58:793.

Macenbbin AH. DNA adduct analysis in fish: laboratory and field studies. In: Malins DC, Ostrander GK, eds. *Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspectives*. Boca Raton, FL: Lewis Publishers; 1994:267–294.

Mackay D. Correlation of bioconcentration factors. *Environ Sci Technol.* 1982;16:274.

MAFF (Ministry of Agriculture, Forestry and Fisheries). *Guidance on Toxicological Study Data for Application of Pesticide Registration*. No. 59-Nousan-4200. Japan: Ministry of Agriculture, Forestry and Fisheries; 1985.

Magurran AE. *Ecological Diversity and its Measurement*. Princeton, NJ: Princeton University Press; 1988.

Malins DC, Anderson KM, Stegeman JJ, et al. Biomarkers signal contaminant effects on the organs of English sole (*Parophrys vetulus*) from Puget Sound. *Environ Health Perspect.* 2006;114:823–829.

Malins DC, Krahn MM, Brown DW, et al. Toxic chemicals in marine sediment and biota from Mukilteo, Washington: relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). *J Natl Cancer Inst Monogr.* 1987;74:487–494.

Mallet J. The evolution of insecticide resistance: have the insects won? *Trends Ecol Evol.* 1989;14:336.

Martineau D, Lemberger K, Dallaire A, et al. Cancer in wildlife, a case study: beluga from the St. Lawrence estuary, Quebec, Canada. *Environ Health Perspect.* 2002;110:285–292.

Mason AZ, Nott JA. The role of intracellular biomineralized granules in the regulation and detoxification of metals in gastropods with special reference to the marine prosobranch *Littorina littorea*. *Aquat Toxicol.* 1981;1:239.

Mathews AP. The relation between solution tension, atomic volume, and the physiological action of elements. *Am J Physiol.* 1904;10:290.

Mauer BA, Holt RD. Effects of chronic pesticide stress on wildlife populations in complex landscapes: processes at multiple scales. *Environ Toxicol Chem.* 1996;15:420.

Mayer LM, Chen Z, Findlay RH, et al. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. *Environ Sci Technol.* 1996;30:2641.

McDonald BG, Chapman PM. PAH phototoxicity—an environmentally irrelevant phenomenon? *Mar Pollut Bull.* 2002;44:1321–1326.

McMahon G, Huber LJ, Moore MJ, Stegeman JJ, Wogan GN. Mutations in c-Ki-ras oncogenes in diseased livers of winter flounder from Boston Harbor. *Proc Natl Acad Sci U S A.* 1990;87:841–845.

Mellanen P, Soimasuo M, Holmbloom B, Oikari A, Santti R. Expression of the Vitellogenin gene in the liver of juvenile whitefish (*Coregonus lavaretus* L. s.l.) exposed to effluents from pulp and paper mills. *Ecotoxicol Environ Saf.* 1999;43:133–137.

Meyer JN, Nacci D, Di Giulio RT. Cytochrome P4501A (CYP1A) in killifish (*Fundulus heteroclitus*): heritability of altered expression and relationship to survival in contaminated sediments. *Toxicol Sci.* 2002;68:69–81.

Meyer JN, Smith JD, Winston GW, Di Giulio RT. Antioxidant defenses in killifish (*Fundulus heteroclitus*) exposed to contaminated sediments and model prooxidants: short-term and heritable responses. *Aquat Toxicol.* 2003;65:377.

Miller KA, Assuncao MGL, Dangerfield NJ, Bandiera SM, Ross PS. Assessment of cytochrome P450 1A in harbour seals (*Phoca vitulina*)

using a minimally-invasive biopsy approach. *Mar Environ Res.* 2005;60:153–169.

Mineau P, ed. *Cholinesterase-Inhibiting Insecticides: Their Impact on Wildlife and the Environment*. Amsterdam, The Netherlands: Elsevier; 1991.

Mineau P, Fletcher MR, Glaser LC, et al. Poisoning of raptors with organophosphorus and carbamate pesticides with emphasis on Canada, US and UK. *J Raptor Res.* 1999;33:1–37.

Miranda ML, Mohai P, Bus J, et al. Policy concepts and applications. In: Di Giulio RT, Benson WH, eds. *Interconnections between Human Health and Ecological Integrity*. Pensacola, FL: SETAC Press; 2002.

Moller P. The alkaline comet assay: towards validation in biomonitoring of DNA damaging exposures. *Basic Clin Pharmacol Toxicol.* 2006;98:336–345.

Mommsen TP, Moon TW. *Environmental Toxicology. Biochemistry and Molecular Biology of Fishes*. Vol. 6. Amsterdam, The Netherlands: Elsevier; 2005.

Moore MN, Allen JI, McVeigh A. Environmental prognostics: an integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. *Mar Environ Res.* 2006;61:278–304.

Mulvey M, Newman MC, Vogelbein W, Unger MA. Genetic structure of *Fundulus heteroclitus* from PAH-contaminated and neighboring sites in the Elizabeth and York Rivers. *Aquat Toxicol.* 2002;61:195.

Mulvey M, Newman MC, Vogelbein W, Unger MA, Ownby DR. Genetic structure and mtDNA diversity of *Fundulus heteroclitus* populations from PAH-contaminated and neighboring sites. *Environ Toxicol Chem.* 2003;22:671.

Munkittrick KR, Portt CB, van der Kraak GJ, et al. Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. *Can J Fish Aquat Sci.* 1991;48:1371–1380.

Myers MS, Stehr CM, Olson OP, et al. Relationships between toxicopathic hepatic lesions and exposure to chemical contaminants in English sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*) from selected marine sites on the Pacific coast, USA. *Environ Health Perspect.* 1994;102:200.

National Research Council. *Risk Assessment in the Federal Government: Managing the Process*. Washington, DC: National Academy of Sciences; 1983.

Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y, Dalton TP. Role of the aromatic hydrocarbon receptor and [*Ah*] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochem Pharmacol.* 2000;59:65–85.

Neely WK, Branson DR, Blau GE. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. *Environ Sci Technol.* 1974;8:1113.

Newman MC. *Quantitative Methods in Aquatic Ecotoxicology*. Boca Raton, FL: CRC/Lewis Press; 1995.

Newman MC. *Population Ecotoxicology*. Chichester, UK: Wiley; 2001.

Newman MC, Crane M, Holloway G. Does pesticide risk assessment in the European Union assess long-term effects? *Rev Environ Toxicol.* 2006;187:1.

Newman MC, Unger MA. *Fundamentals of Ecotoxicology*. 2nd ed. Boca Raton, FL: Lewis/CRC Publishers; 2003.

Nigro M, Falleni A, Del Barga I, et al. Cellular biomarkers for monitoring estuarine environments: transplanted versus native mussels. *Aquat Toxicol.* 2006;77:339–347.

Nott JA, Nicolaidou A. Bioreduction of zinc and manganese along a molluscan food chain. *Comp Biochem Physiol.* 1993;104A:235.

O'Connor RJ. Toward the incorporation of spatiotemporal dynamics into ecotoxicology. In: Rhodes OE Jr, Chesser RK, Smith MH, eds. *Population Dynamics in Ecological Space and Time*. Chicago, IL: The University of Chicago Press; 1996:281.

Odum EP. Trends expected in stressed ecosystems. *Bioscience.* 1985;35:419.

OECD (Organisation for Economic Co-Operation and Development). *OECD Guidelines for the Testing of Chemicals*. Paris, France: OECD Publishing; 1981.

Ohlendorf HM. The birds of Kesterson Reservoir: a historical perspective. *Aquat Toxicol.* 2002;57:1–10.

O'Keefe MK, Baum A. Perceptions of ecosystem health, stress and human well-being. In: Di Giulio RT, Monosson E, eds. *Interconnections between Human and Ecosystem Health*. London: Chapman and Hall; 1996.

Ormseth OA, Ben-David M. Ingestion of crude oil: effects on digesta retention times and nutrient uptake in captive river otters. *J Comp Physiol B.* 2000;170:419.

Ostrander GK, Rotchell JM. Fish models of carcinogenesis. In: Mommsen TP, Moon TW, eds. *Environmental Toxicology, Biochemistry and Molecular Biology of Fishes*. Vol. 6. Amsterdam, The Netherlands: Elsevier; 2005.

Ownby D, Newman MC, Mulvey M, Unger M, Vogelbein W. Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote contaminated sediments. *Environ Toxicol Chem.* 2002;21:1897.

Pacheco M, Santos MA. Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). *Ecotoxicol Environ Saf.* 2002;53:331–347.

Paerl HW, Pinckney JL, Fear JM, Peierls BL. Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse river estuary, North Carolina, USA. *Mar Ecol Prog Ser.* 1999;166:17–25.

Parker ML, Goldstein MI. Differential toxicities of organophosphate and carbamate insecticides in the nestling European starling (*Sturnus vulgaris*). *Arch Environ Contam Toxicol.* 2000;39:233–242.

Partridge GG. Relative fitness of genotypes in a population of *Rattus norvegicus* polymorphic for warfarin resistance. *Heredity.* 1979;43:239.

Patrick R. Use of algae, especially diatoms in the assessment of water quality. *ASTM Spec Tech Bull.* 1973;528:76.

Peakall D. *Animal Biomarkers as Pollution Indicators*. New York: Chapman and Hall; 1992.

Peeters ETHM, Gardeniers JJP, Koelmans AA. Contribution of trace metals in structuring *in situ* macroinvertebrate community composition along a salinity gradient. *Environ Toxicol Chem.* 2000;19:1002.

Peterson CH, Rice SD, Short JW, Esler D, Bodkin BE, Irons DB. Long-term ecosystem response to the Exxon Valdez oil spill. *Science.* 2003;302:2082–2086.

Phillips DH. Detection of DNA modifications by the ^{32}P postlabelling assay. *Mutat Res.* 1997;178:1–12.

Pinkney AE, Harshbarger JC, May EB, Melancon MJ. Tumor prevalence and biomarkers of exposure in brown bullheads (*Ameiurus nebulosus*) from the tidal Potomac River, USA, watershed. *Environ Toxicol Chem.* 2001;20:1196–1205.

Ploch SA, King LC, Kohan MJ, Di Giulio RT. Comparative *in vitro* and *in vivo* benzo(*a*)pyrene DNA adduct formation and its relationship to CYP1A activity in two species of Ictalurid catfish. *Toxicol Appl Pharmacol.* 1998;149:90–98.

Porte C, Janer G, Lorusso LC, et al. Endocrine disruptors in marine organisms: approaches and perspectives. *Comp Biochem Physiol.* 2006;143C:303–315.

Pratt JR, Cairns J Jr. Ecotoxicology and the redundancy problem: understanding effects on community structure and function. In: Newman MC, Jagoe CH, eds. *Ecotoxicology. A Hierarchical Treatment*. Boca Raton, FL: CRC/Lewis; 1996:347.

Preston BL. Indirect effects in aquatic ecotoxicology: implications for ecological risk assessment. *Environ Manage.* 2002;29:311.

Rambler MB, Margulis L, Fester R. *Global Ecology. Towards a Science of the Biosphere*. Boston: Academic Press; 1989.

Rand GM. Behavior. In: Rand GM, Petrocelli AR, eds. *Fundamentals of Aquatic Toxicology*. Washington, DC: Hemisphere; 1985:221–263.

Rands MRW. Pesticide use on cereals and the survival of grey partridge *Perdix perdix* chicks in a field experiment. *J Appl Ecol.* 1985;22:49.

Reichert WL, Myers MS, Peck-Miller K, et al. Molecular epizootiology of genotoxic events in marine fish: linking contaminant exposure, DNA damage, and tissue-level alterations. *Mutat Res Rev Mutat Res.* 1998;411:215–225.

Reinfelder JR, Fisher NS. The assimilation of elements ingested by marine copepods. *Science.* 1991;251:794.

Rice CD, Schlenk D. Immune function and cytochrome P4501A activity after acute exposure to 3,3,4,4,5-pentachlorobiphenyl (PCB 126) in channel catfish. *J Aquat Anim Health.* 1995;7:195–204.

Richardson CJ, Di Giulio RT, Tandy NE. *Free Radical Mediated Processes as Markers of Air Pollution Stress in Trees. Biologic Markers of Air-Pollution Stress and Damage in Forests*. Washington, DC: National Academy Press; 1989:251–260.

Rogers JT, Richards JG, Wood CM. Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol*. 2003;64:215–234.

Ross P, De Swart R, Addison R, et al. Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? *Toxicology*. 1996;112:157–169.

Rotchell JM, Ostrander GK. Molecular markers of endocrine disruption in aquatic organisms. *J Toxicol Environ Health B*. 2003;6:453–495.

Ruiz JM, Barreiro R, Gonzalez JJ. Biomonitoring organotin pollution with gastropods and mussels. *Mar Ecol Prog Ser*. 2005;287:169–176.

Salminen J, Van Gestel CAM, Oksanen J. Pollution-induced community tolerance and functional redundancy in a decomposer food web in metal-stressed soil. *Environ Toxicol Chem*. 2001;20:2287.

Sanchez-Dardon J, Voccia I, Hontela A, et al. Immunomodulation by heavy metals tested individually or in mixtures in rainbow trout (*Oncorhynchus mykiss*) exposed *in vivo*. *Environ Toxicol Chem*. 1999;18:1492–1497.

Sandahl JF, Baldwin DH, Jenkins JJ, Scholtz NL. Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environ Toxicol Chem*. 2005;24:136–145.

Saucier D, Astic L, Rioux P. The effects of early chronic exposure to sublethal copper on the olfactory discrimination of rainbow trout, *Oncorhynchus mykiss*. *Environ Biol Fishes*. 1991;30:345–351.

Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med*. 2001;30:1191–1212.

Schlenk D, Benson W. *Target Organ Toxicity in Marine and Freshwater Teleosts*. New York: Taylor and Francis; 2001.

Schlenger JJ, Stegeman JJ. Induction and suppression of cytochrome P450 1A by 3,3,4,4,5-pentachlorobiphenyl and its relationship to oxidative stress in the marine fish scup (*Stenotomus chrysops*). *Aquat Toxicol*. 2001;52:101–115.

Schmidt CW. Toxicogenomics. *Environ Health Perspect*. 2002;110: A750–A755.

Schmitt CJ, Whyte JJ, Brumbaugh WG, Tillitt DE. Biochemical effects of lead, zinc, and cadmium from mining on fish in the Tri-States district of northeastern Oklahoma, USA. *Environ Toxicol Chem*. 2005;24:1483–1495.

Scholz NL, Truelove NK, French BL, et al. Diazinon disrupts antipredator and homing behaviors in Chinook salmon (*Oncorhynchus tshawytscha*). *Can J Fish Aquat Sci*. 2000;57:1911–1918.

Schwacke LH, Voit EO, Hansen LJ, et al. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the southeast United States coast. *Environ Toxicol Chem*. 2002;21:2752–2764.

Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquat Toxicol*. 2004;68:369–392.

Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL. Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. *Environ Health Perspect*. 1996;104:1296–1300.

Sherratt TN, Roberts G, Williams P, et al. A life-history approach to predicting the recovery of aquatic invertebrate populations after exposure to xenobiotic chemical. *Environ Toxicol Chem*. 1999;18:2512.

Shugart L. DNA damage as a biomarker of exposure. *Ecotoxicology*. 2000;9:329–340.

Sies H, Cadenas E. Oxidative stress: damage to intact cells and organs. *Philos Trans R Soc Lond B Biol Sci*. 1985;311:617–631.

Simkiss K, Daniels S, Smith RH. Effects of population density and cadmium toxicity on growth and survival of blowflies. *Environ Pollut*. 1993; 81:41.

Simonich SL, Hites RA. Global distribution of persistent organochlorine compounds. *Science*. 1995;269:1861.

Sinnhuber RO, Hendricks JD, Wales JH, Putnam GB. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. *Ann N Y Acad Sci*. 1977;298:389–408.

Smith GM, Weis JS. Predator–prey relationships in mummichogs (*Fundulus heteroclitus* (L.)): effects of living in a polluted environment. *J Exp Mar Biol Ecol*. 1997;209:75–87.

Snape JR, Maund SJ, Pickford DB, Hutchinson TH. Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology. *Aquat Toxicol*. 2004;67:143–154.

Sokolova IM. Cadmium effects on mitochondrial function are enhanced by elevated temperatures in a marine poikilotherm, *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). *J Exp Biol*. 2004;207:2639–2684.

Sol SY, Johnson LL, Horness BH, Collier TK. Relationship between oil exposure and reproductive parameters in fish collected following the Exxon Valdez oil spill. *Mar Pollut Bull*. 2000;40:1139–1147.

Solenska M, Micieta K, Misik M. Plant bioassays for an in situ monitoring of air near an industrial area and a municipal solid waste—Zilina (Slovakia). *Environ Monit Assess*. 2006;115:499–508.

Solomon KR, Baker DB, Richards RP, et al. Ecological risk assessment of atrazine in North American surface waters. *Environ Toxicol Chem*. 1996;15:31–76.

Solomon KR, Sibley P. New concepts in ecological risk assessment: where do we go from here? *Mar Pollut Bull*. 2002;44:279.

Spitsbergen JM, Walker MK, Olson JR, Peterson RE. Pathological alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachloro-*para* dioxin as fertilized eggs. *Aquat Toxicol*. 1991;19:41–71.

Spitzer PR, Risebrough RW, Walker W II, et al. Productivity of ospreys in Connecticut–Long Island increases as DDE residues decline. *Science*. 1978;202:333.

Spromberg JA, John BM, Landis WG. Metapopulation dynamics: indirect effects and multiple distinct outcomes in ecological risk assessment. *Environ Toxicol Chem*. 1998;17:1640.

Spry DJ, Wood CM. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. *Can J Fish Aquat Sci*. 1985;42:1332–1341.

Steinert SA. DNA damage as a bivalve biomarker. *Biomarkers*. 1999;4:492–496.

Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses*. Duluth, MN: US EPA; 1985.

Strandberg B, Axelsen JA, Pedersen MB, Jensen J, Attrill MJ. Effect of a copper gradient on plant community structure. *Environ Toxicol Chem*. 2006;25:743.

Sumpter JP, Jobling S. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environ Health Perspect*. 1995;103(suppl 7):173–178.

Tanaka Y, Nakanishi J. Life history elasticity and the population-level effect of *p*-nonylphenol on *Daphnia galeata*. *Ecol Res*. 2001;16:41.

Tanguay RL, Andreasen EA, Walker MK, Peterson RE. Dioxin toxicity and aryl hydrocarbon receptor signaling in fish. In: Schecter A, Gasiewicz TA, eds. *Dioxins and Health*. Hoboken, NJ: Wiley; 2003: 603–628.

Teraoka H, Dong W, Tsujimoto Y, et al. Induction of cytochrome P450 1A is required for circulation failure and edema by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in zebrafish. *Biochem Biophys Res Commun*. 2003;304: 223–228.

Tessier A, Campbell PGC, Auclair JC, Bisson M. Relationships between partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. *Can J Fish Aquat Sci*. 1984;41:1463.

Thomann RV. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ Sci Technol*. 1989;23:699.

Tilman D. Biodiversity: population versus ecosystem stability. *Ecology*. 1996;77:350.

Tilman D, Wedin D, Knops J. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*. 1996;379:718.

Trimble EL. A guest editorial: update on diethylstilbestrol. *Obstet Gynecol Surv*. 2001;56:187–189.

Truhaut R. Ecotoxicology: objectives, principles and perspectives. *Ecotoxicol Environ Saf*. 1977;1:151.

US EPA OPPTS. *OPPTS Harmonized Guidelines, Series 850—Ecological Effects Test Guidelines*. Washington, DC: Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency; 1996a.

US EPA OPPTS. *Ecological Effects Test Guidelines, OPPTS 850.1000. Special Considerations for Conducting Aquatic Laboratory Studies*. EPA 712-C-96-113. Washington, DC: Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency; 1996b.

van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment. *Environ Toxicol Pharmacol*. 2003;13:57–149.

Van Metre PC, Mahler BJ. Trends in hydrophobic organic contaminants in urban and reference lake sediments across the United States, 1970–2001. *Environ Sci Technol*. 2005;39:5567–5574.

Vethaak ADT. Fish disease as a monitor for marine pollution: the case of the North Sea. *Rev Fish Biol Fish*. 1992;2:1–32.

Vogelbein WK, Fournie JW, Van Veld PA, Huggett RJ. Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. *Cancer Res*. 1990;50:5978–5986.

Vos JG, Bossart G, Fournier M, O'Shea T. *Toxicology of Marine Mammals*. New York: Taylor and Francis; 2002.

Walker CH. Neurotoxic pesticides and behavioural effects upon birds. *Ecotoxicology*. 2003;12:307–316.

Wania DM, Mackay D. Tracking the distribution of persistent organic pollutants. *Environ Sci Technol*. 1996;30:390A.

Wania DM, Mckay D, Li Y-F, Bidleman TF, Strand A. Global chemical fate of α-hexachlorocyclohexane. 1. Evaluation of a global distribution model. *Environ Toxicol Chem*. 1999;18:1390.

Wassenberg D, Nerlinger A, Battle L, Di Giulio R. Effects of the PAH heterocycles, carbazole and dibenzothiophene, on *in vivo* and *in vitro* CYP1A activity and PAH-derived embryotoxicity. *Environ Toxicol Chem*. 2005;24:2526–2532.

Wassenberg DM, Di Giulio RT. Synergistic embryotoxicity of polycyclic aromatic hydrocarbon aryl hydrocarbon receptor agonists with cytochrome P4501A inhibitors in *Fundulus heteroclitus*. *Environ Health Perspect*. 2004a;112:1658–1664.

Wassenberg DM, Di Giulio RT. Teratogenesis in *Fundulus heteroclitus* embryos exposed to a creosote-contaminated sediment extract and CYP1A inhibitors. *Mar Environ Res*. 2004b;58:163–168.

Watanabe H, Iguchi T. Using ecotoxicogenomics to evaluate the impact of chemicals on aquatic organisms. *Mar Biol*. 2006;149:107–115.

Wayland M, Bollinger T. Lead exposure and poisoning in bald eagles and golden eagles in the Canadian prairie provinces. *Environ Pollut*. 1999;104:341.

Webb NA, Wood CM. Physiological analysis of the stress response associated with acute silver nitrate exposure in freshwater rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem*. 1998;17:679–688.

Webb RE, Horsfall F Jr. Endrin resistance in pine mouse. *Science*. 1967;156:1762.

Webber HM, Haines TA. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). *Environ Toxicol Chem*. 2003;22:1556–1561.

Weinstein JE, Oris JT, Taylor DH. An ultrastructural examination of the mode of UV-induced toxic action of fluoranthene in the fathead minnow, *Pimephales promelas*. *Aquat Toxicol*. 1997;39:1–22.

Wells RS, Tornero V, Borrell A, et al. Integrating potential life-history and reproductive success data to examine relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. *Sci Total Environ*. 2005;349:106–119.

Wendelaar Bonga SE, Lock RAC. Toxicants and osmoregulation in fish. *Neth J Zool*. 1992;42:478–493.

Wendelaar Bonga SE, Lock RAC. The osmoregulatory system. In: Di Giulio RT, Hinton DE, eds. *The Toxicology of Fishes*. CRC Press. In press.

Wester PW, van der Ven LTM, Vethaak AD, Grinwis GCM, Vos JG. Aquatic toxicology: opportunities for enhancement through histopathology. *Environ Toxicol Pharmacol*. 2002;11:289–295.

Weston DP, Mauuya KA. Predicting bioavailability and bioaccumulation with in vitro digestive fluid extraction. *Environ Toxicol Chem*. 2002;21:962.

Wiese FK, Robertson GJ. Assessing seabird mortality from chronic oil discharges at sea. *J Wildl Manage*. 2004;68:627–638.

Wilson L, Martin PA, Elliot JE, Mineau P, Cheng KM. Exposure of California quail to organophosphorus insecticides in apple orchards in the Okanagan Valley, British Columbia. *Ecotoxicology*. 2001;10:79–90.

Winston GW, Adams SM, Benson WH, et al. Biological bases of similarities and differences. In: Di Giulio RT, Benson WH, eds. *Interconnections between Human Health and Ecological Integrity*. Pensacola, FL: SETAC Press; 2002.

Winston GW, Di Giulio RT. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquat Toxicol*. 1991;19:137–161.

Wirgin I, Currie D, Garte SJ. Activation of the K-ras oncogene in liver tumors of Hudson River tomcod. *Carcinogenesis*. 1989;10:2311–2315.

Woin P. Short- and long-term effects of the pyrethroid insecticide fenvalerate on an invertebrate pond community. *Ecotoxicol Environ Saf*. 1998;41:137.

Wolfe MF, Schwarzbach S, Sulaiman RA. Effects of mercury on wildlife: a comprehensive review. *Environ Toxicol Chem*. 1998;17:146–160.

Woodward M. *Epidemiology. Study Design and Data Analysis*. 2nd ed. Boca Raton, FL: Chapman & Hall/CRC; 2005.

Wootton EC, Dyrynda EA, Ratcliffe NA. Comparisons of PAH-induced immunomodulation in three bivalve molluscs. *Aquat Toxicol*. 2003;65:13–25.

Wu RSS. Hypoxia: from molecular responses to ecosystem response. *Mar Pollut Bull*. 2002;45:35–45.

Ylitalo GM, Stein JE, Hom T, et al. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). *Mar Pollut Bull*. 2005;50:30–39.

Zelikoff JT, Carlson E, Li Y, et al. Immunotoxicity biomarkers in fish: development, validation and application for field studies and risk assessment. *Hum Ecol Risk Assess*. 2002;8:253–263.