Biomonitoring in the Water Environment

A Special Publication

Prepared by **Task Force on Biomonitoring in the Water Environment**
Robin Zimmer, *Chair*

Max L. Anderson
John G. Aronson
Greg R. Bright
Jim Buckles
Dave N. Commons
G. Michael De Graeve
Elie M. Dick
William E. Foster
Roger R. Hlavek
Carolyn T. Hunsaker
Martin J. Lipschultz
Charles L. Logue
R.D. Roop
Brian A. Wade

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Principal authors of this manual are

Preface	Carolyn T. Hunsaker
Chapter 1	Billy Isom and Carolyn T. Hunsaker
Chapter 2	Greg R. Bright
Chapter 3	Martin J. Lipschultz, William E. Foster, and Lynn Adams Kszos
Chapter 4	Robin Zimmer
Chapter 5	Carolyn T. Hunsaker, Kenneth Dickson, William Waller, and E. Morgan
Chapter 6	Robin Zimmer
Chapter 7	Greg R. Bright and Roxanna Hinzman

Contributing authors are

John G. Aronson
Jeffrey Black
Don Blancher
G. Michael De Graeve
Anwar Khan
Wayne McCulloch

Additional review was provided by Dave Beeson.

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Advanced Aquatic Technology Associates, Fort Collins, Colorado
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For information on membership, publications, and conferences, contact

Water Environment Federation
601 Wythe Street
Alexandria, VA 22314-1994 USA
1-703-684-2400

Water Environment Federation Technical Practice Committee Control Group

L.J. Glueckstein, *Chair*
T. Popowchak, *Vice-Chair*

G.T. Daigger
P.T. Karney
T.L. Krause
J. Semon
R. Zimmer

Authorized for Publication by the Board of Directors
Water Environment Federation

Quincalee Brown, *Executive Director*

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Preface

While biomonitoring and associated research have been ongoing for many decades, the publication of *Technical Support Document for Water Quality-Based Toxics Control* (U.S. EPA, 1985—see reference list in Chapter 1) was probably the single most important event in recent time in structuring biomonitoring as most people in the water quality field think of it today. Through workshops and conference sessions since 1984, the Ecology Committee of the Water Environment Federation has been working to alert and educate its members and the community involved in water pollution prevention about regulatory developments and scientific issues related to biomonitoring. From these activities, the Ecology Committee was convinced that a primer on biomonitoring in the water environment was needed to provide guidance to the broad set of disciplines (engineering, ecology, toxicology, chemistry, environmental science, and law) involved in protecting aquatic ecosystems. Hence, the purpose and structure of this primer are to provide general information about the following aspects of biomonitoring: history and need, the regulatory framework, biological criteria, standard test methods, quality assurance and control, toxicity reduction evaluations, and the use of testing and monitoring data in assessments and policy making. Although this book provides the reader with basic information on biomonitoring, it is not a methods manual; rather, it directs the reader to additional literature for more in-depth information. The primary audience is intended to be water quality professionals without extensive training in the biological sciences. We hope it will be useful to a broad set of professionals and especially to those people encountering biomonitoring for the first time. It should also be useful as a textbook at both the undergraduate and graduate levels.

Chapter 1 provides a historical overview of biomonitoring and background on the regulatory authority that implements current biomonitoring requirements. Biomonitoring is indispensable to the development of criteria for protecting the water environment because almost all criteria are designed to protect some biological system—aquatic life, wildlife, or human health. Chapter 2 includes discussions of the use of biomonitoring to develop criteria, important issues one should be aware of about the development and use of criteria, and the current status and possible future trends of criteria. Chapter 3 discusses standard freshwater and saltwater toxicity tests. The methods for the statistical interpretation of data and use of endpoints for permit compliance are explained. Procedures are given for sample procurement, sample manipulation, and organism handling for the freshwater tests. Chapter 3 also briefly discusses sediment and mesocosm bioassay testing. The importance of accurate biomonitoring data cannot be overemphasized because the data are used in permit and enforcement activities. Chapter 4 provides information to assist

the reader in evaluating the performance of bioassay laboratories as well as the quality and defensibility of aquatic toxicity data.

Understanding and effectively managing aquatic ecosystems at the watershed/drainage basin level have long been objectives of ecologists. Chapter 5 addresses issues and current activities and technology that are moving us closer to that goal. The ability to measure real-time, in-stream episodic toxicity is discussed. An overview of ecological risk assessment is given in Chapter 5, along with examples of government monitoring and assessment programs for water quality at the watershed and regional scales. Where periodic monitoring indicates the continuing presence of unacceptable levels of toxicity in a permitted discharge, regulatory authorities require implementation of a plan to identify the causative factors and subsequently reduce or eliminate the wastewater toxicity. This effort is called a *toxicity reduction evaluation*, and its concepts and approaches are described in Chapter 6. Case studies are provided in Chapter 7 to familiarize the reader with the variety of approaches used in biomonitoring studies and programs. Subjects include a municipal discharger that performed copper and mercury studies and an industrial discharger that performed whole effluent and sediment toxicity tests. A watershed study for a wildlife management area illustrates the use of biomonitoring for distinguishing toxicity from irrigation water versus that from other sources. An overview of the Biological Monitoring and Abatement Program at the Y-12 Plant in Oak Ridge, Tennessee, is presented to illustrate the effectiveness of integrated ecological monitoring in evaluating the recovery of disturbed systems. Data are included for the following tasks: toxicity testing; bioindicator studies; community studies of periphyton, benthic macroinvertebrates, and fish; bioaccumulation of contaminants in aquatic and terrestrial biota; and data management. A final case study explains the use(s) of biological indicators in evaluating aquatic ecosystem health and determining human health risks from aquatic systems.

Chapter 1
History of and Need for Biomonitoring

1 **Terminology**
2 **Historical Overview**
3 **Biomonitoring Today**
6 **Regulatory Authority and Current Implementation of Biomonitoring**
7 **References**

TERMINOLOGY

Historically, the terms *bioassay*, *toxicity testing*, and *biological monitoring* (or *biomonitoring*) had well-defined meanings. However, today they are no longer well-defined in the literature. To distinguish the differences between the terms, definitions are provided here. *Bioassay* is a determination of the relative strength or toxicity of a drug or chemical by observing a selected test organism's response. Bioassays were largely used for such determinations in the absence of analytical technologies. "A *toxicity test* measured the degree of response of an exposed test organism to a specific chemical or effluent" (U.S. EPA, 1985). The term *biomonitoring* (biological monitoring), historically reserved to mean the determination of the biological integrity of surface waters, was defined as part of the National Water Quality Network in 1956 under Section 4(c) of the Federal Water Pollution Control Act (PL 84-660). It was legally defined in Section 502(15) of the 1972 and 1977 Federal Water Pollution Control Act Amendments (PL 92-500 and PL 95-217, respectively) and the Clean Water Act of 1987 (PL 100-4):

> The term "biological monitoring" shall mean the determination of the effects on aquatic life, including accumulation of pollutants in tissue, in receiving waters due to the discharge of pollutants (A) by techniques and procedures, including sampling of organisms representative of appropriate levels of the food chain appropriate to the volume and the physical, chemical, and biological characteristics of the effluents, and (B) at appropriate frequencies and locations.

Biomonitoring (biological monitoring) encompasses toxicity testing; ecological characterization such as population surveys of periphyton, benthic macroinvertebrates, and fish communities; bioaccumulation of contaminants; and biological indicators such as overall fish health and reproductive status. This book primarily focuses on toxicity testing, although Chapter 5 discusses monitoring for impact assessment and biological indicators.

HISTORICAL OVERVIEW

A brief review of the history of biomonitoring is given here. As of 1980, Weber (1973 and 1980) provided both a comprehensive review of federal and state biomonitoring programs based on legislation and a chapter on regulatory control and public policy. Both toxicity test and ambient biomonitoring test selection, application, and interpretation of data were addressed. Anderson (1980) discussed the historical use of aquatic invertebrates for toxicity testing dating back to the early 1800s. Hunn (1989) discussed the historical use of acute toxicity tests with fish from 1863 to 1987. These references with their citations provide an understanding of the history of toxicity testing; however, none provides insight to the historical setting leading to the present use of toxicity testing and biomonitoring procedures.

Federal and state biomonitoring as we know it today developed in the 1950s and 1960s largely from the efforts of the U.S. Environmental Protection Agency's (U.S. EPA's) predecessor: the U.S. Public Health Service, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. This center developed from the Stream Pollution Investigations Station established in Cincinnati in 1913.

In the 1950s, the center developed a technical transfer and training program in which engineers, scientists, biologists, and chemists of different specialties provided instruction courses in ecology and chemistry of water pollution. For example, a course at the Taft Sanitary Engineering Center in 1958 included information about biological indicators of organic enrichment, evaluation of toxic substances and biological agents in water supplies, and biological practices in water quality management. Within these categories, definitive lectures included "The Interpretation of Biotic Responses to Pollution," "An Assessment of Pollution Damage to Aquatic Biota," "Algae as Indicators of

Pollution," and "Fundamentals of the Toxicity Bioassay" (U.S. Public Health Service, 1958). These courses were directed primarily at states for training of personnel to implement, expand, or improve water pollution control efforts. Biomonitoring and toxicity testing were significant parts of the training conducted by the center, through which many professionals were initiated to this field. Biomonitoring techniques development and use continued in the U.S. throughout the late 1950s and 1960s primarily in the absence of regulatory permitting requirements. Simultaneously, Sprague (1969, 1970, and 1971) consolidated the use of toxicity testing on measurement of pollutant toxicity to fish: methods, application of results, and sublethal effect and "safe" concentrations (Buikema and Voshell, 1993).

Concurrent with the biomonitoring standardization efforts of U.S. EPA in the 1970s, such as those of Weber (1973) and Peltier (1978), ASTM was actively developing methods and supporting symposia for technical support of biomonitoring, such as Cairns and Dickson (1973) and Bates and Weber (1981). Furthermore, the value of methods issued by the American Public Health Association since 1970 cannot be underestimated, especially to water quality professionals and wastewater treatment plant operators (for example, see APHA, 1995).

BIOMONITORING TODAY

Despite all of the research and application of biomonitoring methods before 1984, in particular toxicity testing, the practice was not taken seriously in the regulatory community until U.S. EPA issued its national policy on toxics control (U.S. EPA, 1984). The following was included in the statement of policy: "In addition to enforcing specific numerical criteria, EPA, and the States will use biological techniques and available data on chemical effects to assess toxicity impacts and human health hazards based on the general standard of 'no toxic materials in toxic amounts.'" The policy further stated

> To carry out this policy, EPA Regional Administrators will assure that each Region has the capability to conduct water quality assessments using both biological and chemical methods and provide technical assistance to the States.

The National Pollutant Discharge Elimination System (NPDES) began in 1977. In September 1985, U.S. EPA issued perhaps its most formative document implementing toxicity testing in the NPDES permitting process, *Technical Support Document for Water Quality-Based Toxics Control* (U.S. EPA, 1985). This document translated toxicity test results to meaningful values for use in engineering applications, including the toxic unit, which is the "reciprocal of the effluent dilution that causes the acute effect by the end of the acute exposure period." Through this approach, acute and chronic toxic

criteria, unlike toxicity endpoints such as lowest observed effect concentration or median lethal concentration, can be used directly in stream flow and waste load allocation calculations.

While chemical-specific limitations had improved water quality in the U.S. by the 1980s, there was no guarantee that aquatic systems were at the desired condition. U.S. EPA explained the need for biological testing in part as follows (Wall and Hanmer, 1987):

> However, over time, it has become apparent that a chemical-specific approach, by itself, cannot adequately protect all surface waters because many toxic compounds cannot be measured by available detection methods. In addition, toxicological data are unavailable for thousands of toxic compounds that are routinely discharged to surface waters. Finally, data on the effects of individual compounds do not account for the interactions among pollutants that may occur in the complex mixture of toxicants that comprises many industrial and municipal wastewaters.

Toxicity testing activities during the 1970s were given formal impetus in 1981 when the Complex Effluent Toxicity Testing Program was initiated. "EPA determined that toxicity tests conducted on a minimum of three to five test species from a range of taxonomic groups can predict whether or not effluents will adversely effect a receiving water and can be used to control toxic wastewater discharges" (Wall and Hanmer, 1987). Wall and Hanmer noted that, by July 1986, toxicity testing had been written into more than 1 800 NPDES permits in the U.S.

The development of standardized toxicity testing procedures such as Weber and Peltier (1985) helped push the U.S. EPA testing program forward. Methods development, publications by U.S. EPA, and the 1987 Clean Water Act ensured that toxicity bioassay testing would be a regulated requirement for toxicity detection and toxicity control in U.S. wastewater.

Two early examples of the use of bioassays in resolving water pollution problems can be found in Isom (1960) and Mount and Putnicki (1966). Isom's study involved continued unresolved fish kills associated with phosphorus (elemental) reduction plants in middle Tennessee. At the time of the study, the plants had been associated with numerous but periodic fish kills on the Duck River. Waste stream screening tests produced circumstantial evidence that elemental phosphorus was associated with the fish kills, but no analytical methods or instrumentation was available for analysis at the time. Through laboratory bioassays using submicron filters to separate soluble from colloidal

fractions of elemental phosphorus, which was determined to be in the P_4 form found in the fish, Isom was able to demonstrate that elemental phosphorus was the cause of the fish kills. This approach was later confirmed and expanded by Idler (1969) and others in Canada. This set of studies is the basis for the U.S. EPA water quality criterion for elemental phosphorus in marine habitats (U.S. EPA, 1986).

The second example of using bioassays to solve a significant pollution problem was the study by Mount and Putnicki (1966). Through the use of bioassays, field studies, and supporting analytical analyses, Mount and Putnicki were able to identify the cause of significant fish kills occurring on the lower Mississippi River from 1963 to 1964. They established that endrin was present at toxic concentrations in the blood of dying fish and observed the development of an "exact duplication of the toxicity syndrome observed in the...dying river fish including histological, hematological, and behavioral symptoms." They were able to tie potential sources of endrin to a manufacturer in Memphis, Tennessee, and to other sources. This study offered critical proof of the value of using bioassays in combination with field and laboratory studies to solve pollution problems. Soon after this study, the U.S. EPA National Environmental Laboratory System was established, and Mount became Director of the Duluth, Minnesota, facility, where he led extensive biomonitoring research and development.

In another formative study, Walsh and Garnas (1983) used toxicity testing of chemical fractions of liquid wastes with freshwater and saltwater species of algae and crustaceans, thus establishing the conceptual framework for toxicity identification evaluations (TIEs). The TIE approach was then formalized by Mount and his associates at the U.S. EPA laboratory in Duluth, Minnesota (U.S. EPA, 1988), and completed by Norberg-King *et al.* (1991). The NPDES program recognized that toxicity limitations identified toxic wastewater but that causes of toxicity were often unknown. Therefore, the TIE program at the U.S. EPA laboratory in Duluth developed methods to fulfill this need. Subsequently, municipal- and industrial-specific toxicity reduction evaluation procedures and approaches were implemented.

The Complex Effluent Toxicity Testing Program was eliminated with the U.S. government budget cuts of 1993. Current emphasis is on developing biomonitoring toxicity methods and quality criteria for sediment. It remains to be seen if this reduced effort by U.S. EPA will support the goals of "no toxic materials in toxic amounts" in streams receiving wastewater discharges and of the identification of environmental residuals in sediments.

REGULATORY AUTHORITY AND CURRENT IMPLEMENTATION OF BIOMONITORING

The Clean Water Act of 1977 specifies an objective of restoring and maintaining the chemical, physical, and biological integrity of U.S. waters. The act provides broad authority to U.S. EPA for the following activities:

- Establish the NPDES permit program and the national pretreatment program;
- Define pollution control technologies and establish effluent limitations;
- Obtain information through reporting and compliance inspections; and
- Take enforcement actions, civil and criminal, when violations of the act occur.

Through the 1977 Clean Water Act, a federal policy was established that prohibited the discharge of "toxic substances in toxic amounts." While U.S. EPA has been mandated to uphold and enforce this national policy, states are required to work in concert with U.S. EPA in developing programs to ensure compliance.

Addressing the national policy of prohibiting toxic substance discharges in toxic amounts, U.S. EPA outlined the policy objectives for development of NPDES permit limitations (third round) by following the simple approach of best available technology (U.S. EPA, 1984). This policy states

> To control pollutants beyond Best Available Technology Economically Achievable (BAT), secondary treatment, and other Clean Water Technology-based requirements in order to meet state water quality standards, the EPA will use an integrated strategy consisting of both biological and chemical methods to address toxic and nonconventional pollutants from industrial and municipal sources. Where State standards contain numerical criteria for toxic pollutants, NPDES permits will contain limits as necessary to assure compliance with these standards. In addition to enforcing specific numerical criteria, EPA and the States will use *biological techniques* and available data on chemical effects to assess toxicity impacts and human health hazards based on the general standard of "no toxic materials in toxic amounts."
>
> Where violations of water quality standards are identified or projected, EPA and the States will develop water quality based effluent limits for inclusion in any issued permit. Where there is a significant likelihood of toxic effects to biota in the receiving

stream, EPA and the States may impose permit limits on effluent toxicity and may require an NPDES permittee to conduct a toxicity reduction evaluation. Where toxic effects are present but there is a significant likelihood that compliance with technology based requirements will sufficiently mitigate the effects, EPA and the States may require chemical and *toxicity testing* after installation of treatment and may reopen the permit to incorporate additional limitations if needed to meet water quality standards.

Section 122.44(d)(1) of Title 40 of the Code of Federal Regulations requires U.S. EPA and the delegated states to evaluate each NPDES permit for the potential to exceed state numerical or narrative water quality standards, including those for toxics, and establish effluent limits for those facilities with the "reasonable potential" to exceed those standards. These regulations require chemical-specific limits, based on state numerical water quality standards or other criteria developed by U.S. EPA, and whole effluent toxicity limits that use biomonitoring.

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Chapter 2
Criteria for Protecting the Water Environment

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Introduction

The word *criteria* describes specific types of measurements used to decide how to protect beneficial uses of a water body. Criteria have no particular legal status until they are adopted by a regulatory agency to create a standard. A standard consists of both a criterion (the basis for making a decision) and that which must be protected (for example, aquatic life). A particular criterion may be identical in different regulatory jurisdictions but still result in different standards if the uses to be protected are different.

Criteria have been developed for both water and aquatic sediments. The philosophy behind criteria development is changing with each advancement in the environmental sciences of chemistry, toxicology, and ecology. This chapter includes a discussion of how biomonitoring has been used to develop

criteria, problems in criteria development and use, the current status of criteria, and possible future trends.

How has biomonitoring been used to develop criteria?

Biomonitoring is indispensable to criteria development because almost all criteria are designed to protect some biological system (for example, aquatic life, wildlife, or human health). Even though most criteria take the form of a numerical chemical measurement (for example, parts per million copper), that chemical analysis is merely an indirect way of protecting against adverse biological effects. In addition to such *numerical criteria,* some regulatory agencies have also adopted *narrative criteria* to protect water bodies. For example, the narrative criterion "one-tenth the 96-hour lethal concentration for an important freshwater species" can be just as useful as a numerical criterion of "50 ppb copper." The use of *biocriteria* to protect certain designated uses of a water body will be discussed in more detail later.

Studies of the effects of chemicals on aquatic life have been conducted since at least the early 1900s. From the beginning, these studies reported information about the test organism used, the time period studied, and the concentration–effect relationships observed for particular chemicals or wastewater. The first attempt to summarize and disseminate this information on a national level was made by the National Technical Advisory Committee (1968). In 1980, the U.S. Environmental Protection Agency (U.S. EPA) formalized a method for using all of this information to derive criteria. The method has been updated several times since then, but most criteria still depend largely on biomonitoring test results.

Water quality criteria

Since the formalization of a method, U.S. EPA (1986) has published water quality criteria for approximately 150 chemicals. An example of the water quality criteria for hexavalent chromium is presented in Table 2.1. Criteria have been derived to protect aquatic life, prevent potentially harmful levels of bioaccumulation of chemicals, and protect human health from chemicals in drinking water or consumed fish. Recently, there have been attempts to formalize a method to protect wildlife from potential adverse effects of chemicals as well.

U.S. EPA's water quality criteria for aquatic life are designed to provide protection to 95% or more of all species that could be exposed to a particular chemical. All acute and chronic toxicity information available for a particular chemical, in the form of assembled studies that are not conducted with a

Table 2.1 Water quality criteria—hexavalent chromium

Criterion, µg/L	Use to be protected	Methods used
2.4	Freshwater aquatic life (acute exposure)	Aquatic toxicity tests
0.012	Level of mercury in fish[a]	Bioaccumulation tests
2.1	Marine aquatic life (acute exposure)	Aquatic toxicity tests
0.025	Level of mercury in oysters[a]	Bioaccumulation tests
0.000 18	Wildlife	Toxicity from oral exposure

[a] To be kept below U.S. Food and Drug Administration action level.

necessary level of quality assurance, is eliminated, and criteria that should prevent adverse effects to the aquatic community are statistically extrapolated from all remaining test results. Minimum data requirements for this process include toxicity information necessary for an acute criterion, and the information must include data on certain kinds of fish and invertebrates (U.S. EPA, 1986).

There are potential problems associated with this method of criteria development. For example, the toxicity of most chemicals has not been tested adequately to develop criteria with a high degree of confidence. Most biomonitoring tests have been short term (acute tests), using a handful of common test organisms. The ability to extrapolate from this small short-term data set to provide accurate answers about long-term toxicity to an entire aquatic community is limited. A second problem is that the toxicity of many chemicals varies under different test conditions. For example, many pollutants are more toxic in chemically simple dilution waters required by the criteria derivation method than in natural waters containing more complex mixtures of substances. To develop a good criterion under these site-specific circumstances, whereby aquatic life uses are adequately protected without being overly protected, is a continuing challenge.

Although beyond the scope of this book, the development of wildlife and human health criteria also depends on a form of biomonitoring. For example, the wildlife criteria currently being considered in U.S. EPA's Great Lakes Initiative program will depend on laboratory tests of wild or domestic animals to determine potential effects of chemicals on predator wildlife species (for example, ospreys, mink, and otters) (U.S. EPA, 1993). The development of human health effects criteria depends on tests with animals (primarily with laboratory rats) to extrapolate potential adverse effects on humans. Like methods used to develop aquatic life criteria, these wildlife and human health criteria methods will continue to evolve to provide better predictions of how chemicals affect organisms.

SEDIMENT CRITERIA. Until recently, almost all criteria developed to protect water bodies have dealt only with water itself and ignored the environmental consequences of sediment contamination. However, aquatic sediments can be a repository for many pollutants that are relatively insoluble in water or are capable of binding with other sediment constituents and accumulating over time. Biomonitoring tests have often shown that animals exposed to contaminated sediments can be adversely affected, even though the overlying water is clean (Baudo *et al.*, 1990, and Burton [Ed.], 1992). Therefore, the 1987 Clean Water Act amendments began to address management of "in-place" pollutants associated with contaminated sediments (Clean Water Act of 1987, or PL 100-4). Examples of sediment quality criteria for dieldrin are given in Table 2.2 (59 *Federal Register*, 1994).

Table 2.2 Sediment quality criteria—dieldrin

Criterion	Use to be protected	Methods used
11 $\mu g/g_{oc}$[a]	Freshwater benthic life	Equilibrium partitioning + toxicity tests
20 $\mu g/g_{oc}$[a]	Saltwater benthic life	Equilibrium partitioning + toxicity tests
0.000 6 $\mu g/g$	Bioaccumulation	Equilibrium partitioning

[a] These criteria recognize the effect of organic carbon content on the toxicity of the compound (g_{oc} = gram of organic carbon in the sediment).

U.S. EPA currently has no national sediment criteria for pollutants. However, in response to the immediate need to address "in-place pollutants" associated with dredging projects for navigation purposes, several states, federal agencies, and Canadian provinces have developed their own sediment criteria. For example, the Ontario Ministry of the Environment (OME) recently published guidelines for evaluating sediments based on data for approximately 40 chemicals (primarily chlorinated pesticides, polychlorinated biphenyls [PCBs], and polynuclear aromatic hydrocarbons) (Persaud *et al.*, 1992). These criteria were developed using a variety of chemical and biological methods to protect aquatic life and prevent significant bioaccumulation. Where no numerical guidelines exist, the OME approach uses sediment bioassays with fathead minnows and the burrowing mayfly *Hexagenia limbata* to calculate biologically based criteria. The U.S. Army Corps of Engineers uses a similar biological approach to make decisions about disposal of dredge spoils under its jurisdiction. When sediments cause no adverse effects to test organisms, they are typically considered safe for disposal as fill material (U.S. EPA and U.S. ACOE, 1992).

The development of specific numerical sediment criteria has been hampered by the complexity of chemical interactions known to occur in most sediments. For example, many toxicants are known to become easily bound to

organic carbon in sediments, making their potential adverse effects inversely related to organic carbon content. Other studies have shown that acid-volatile sulfide, a natural constituent of most sediments, is also capable of interacting with metals to reduce their toxicity. Because chemical measurements alone have little value in predicting toxicant effects in sediment, a combination of chemical and biological criteria is thought to be necessary. One such technique, called the *sediment quality triad*, uses information about chemistry, laboratory bioassays, and *in situ* bioassessment data to make decisions about sediment quality. U.S. EPA is currently considering another assessment technique called the *apparent effects threshold* (the highest sediment concentration of a contaminant above which an adverse biological effect is always observed) to develop sediment criteria. Baudo *et al.* (1990) and Burton (Ed.) (1992) provide good overviews of sediment toxicity assessment and issues involved with sediment criteria.

BIOACCUMULATION STUDIES. Some chemicals are not particularly toxic to aquatic animals at levels found in most water bodies but instead can be concentrated within the animals' tissues to much higher levels. When this happens, the chemical-contaminated tissues can affect the animals that consume them. For example, nearly all aquatic organisms tolerate mercury in the 0.1-µg/L range without any adverse effects. Under certain conditions, however, mercury can bioaccumulate in exposed animals in up to 50 000 times more than ambient water concentrations. Frequent consumption of organisms with these levels can cause adverse health effects in wildlife or human consumers (McKim *et al.*, 1976).

The ability of certain chemicals to bioaccumulate has resulted in numerous fish consumption advisories in the U.S. and Canada. Some chlorinated pesticides, PCBs, and dioxin, for example, are undetectable in water samples from the Great Lakes using presently available technology but are present in fish at concentrations that make them potentially dangerous to human and wildlife consumers. Therefore, a method to measure bioaccumulation potential is essential in developing criteria for these chemicals.

Biomonitoring techniques have been used to measure the potential of chemicals to accumulate in aquatic animals. Bioaccumulation can occur through two mechanisms: direct uptake from the water or uptake from both water and food. Because of the high expense and difficulty of continuous measurement, there are relatively few studies available in which both food and water sources of uptake are considered. However, many studies of uptake from water alone have been conducted for chemicals (these are more properly called *bioconcentration* studies). The following factors are typically considered essential to develop criteria based on bioaccumulation:

- The tests must be flow-through (the chemical must be continually renewed to prevent degradation over time).

- The concentration of the chemical should be monitored continuously in test water to ensure that little variation has occurred.
- The test must continue until steady-state conditions have been reached (the concentration of chemical in the test animal's tissues no longer increases).
- If the chemical is known to concentrate in lipids (the fatty tissue of animals), percent lipids should be determined. Many chlorinated organic compounds fall into this category.

The organization ASTM published Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks, which covers these test procedures in more detail (ASTM, 1993).

BIOCRITERIA. As discussed previously, numerical criteria for individual chemicals do not always provide the degree of protection for which they were designed. Numerous complicating factors can increase or decrease potential toxic effects predicted by laboratory tests with single chemicals. *Biocriteria* have been proposed as a way to deal with these many complicating factors (U.S. EPA, 1989). A biocriteria approach would substitute a direct biological measurement for an indirect chemical measurement of ecological health.

What kinds of biological measurements of ecological condition or health are available? Chapter 5 describes some of these bioassessment measures in more detail. Typically, such measures are based on a comparison of the biological communities of particular study sites with those of reference sites known to be the best achievable in a given geographic area. Aquatic communities incorporated to these biocriteria measurements include fish, macroinvertebrates, and algae. An example of a biocriterion based on a resident fish community is as follows (U.S. EPA, 1989):

- Criterion: "good" ecological integrity = less than 80% difference between the site score and the "reference."
- Use to be protected: aquatic life.
- Methods used: rapid bioassessment.

Because there are significant differences in aquatic ecosystems in different geographic areas, biocriteria are best applied on a regional rather than national basis. U.S. EPA has helped develop guidelines for biocriteria but has left the responsibility for their implementation to state agencies responsible for protecting water quality. The states of Arkansas, Florida, Maine, and Ohio, for example, have already incorporated some form of biocriteria to their water quality standards. The biocriteria used in Ohio are based on both fish and macroinvertebrate measures and are different for each of the state's five ecoregions. Many other states are currently exploring the use of biocriteria in their water quality decision-making processes.

To be most useful in protecting water quality, biocriteria should satisfy the following requirements:

- They should use some type of biological assessment (for example, an index value based on community structure or function).
- They should be reproducible (different investigators should be able to produce the same general results).
- Variability of the measures must be low (too much variation makes it difficult to distinguish affected from unaffected sites).
- The measures should be sensitive to other organisms not directly involved in the monitoring.
- The measures should be appropriate for the water body being assessed (for example, biocriteria for streams will be different from those used for lakes).

FUTURE TRENDS

In the future, water quality scientists will be examining the following important and unresolved issues, which are briefly discussed, regarding criteria:

- Deciding how to take into account the bioavailable form of pollutants;
- Designing criteria to protect water quality during wet weather;
- Determining how to develop criteria that take into account mixtures of pollutants and possible synergistic effects;
- Establishing criteria that better protect keystone, habitat-forming, and endangered species;
- Developing site-specific criteria that take into account the unique qualities of individual water bodies; and
- Basing criteria on ecological risk, such as making the duration of exposure and allowable frequency of exceeding criteria part of the decision-making process.

In the toxicity tests used by U.S. EPA to develop metals criteria for protection of aquatic life, most of the metal fraction present was added from highly purified salts and was, therefore, present primarily in a dissolved form (Allen and Hansen, 1996). In contrast, some of the metals in the natural environment can become bound to less soluble particulate matter. This is important in measuring metal toxicity because soluble forms are bioavailable (for example, pass through cell membranes), thus are more toxic than complex forms such as organometals. Research is ongoing to determine how much of each metal is typically in the dissolved form and to use this ratio to correct the original criteria. Ward (1996) discusses this issue with two examples for copper and illustrates the water-effects ratio, or WER (ratio of the toxic endpoint in site water

to the toxic endpoint in laboratory water). The WERs for copper in textile effluent were 16 and 39, respectively, in his examples.

All previous criteria were designed to protect the environment from long-term exposure during low flow conditions. Dry weather criteria are an example of these. However, dry weather criteria can be misleading if used to make decisions about short-term exposures to chemicals occurring during wet weather. For example, stormwater runoff may contain chemical concentrations that exceed dry weather criteria. However, because these exposures occur only for a few hours during times when higher flows are present, the effect may be minimal. Wet weather criteria are being developed that will adequately protect the environment while taking these additional factors into account.

In the past, water quality criteria have been derived using toxicity tests in which exposure to only a single chemical occurred. However, little wastewater or stormwater contains only a single chemical. Mixtures of chemicals can cause changes in the toxicity of the individual constituents. For example, two chemicals present in a mixture, each at a concentration only half the amount needed to be lethal, may be as toxic as if the full amount of either one were present. This characteristic is called *additivity.* In some mixtures, the toxicity of individual chemicals may be reduced (*antagonism*) or increased beyond their individual effects (*synergism*) because of various interactions between the chemicals. Because it is inherently difficult to predict how chemicals will interact, developing criteria for chemical mixtures will be a challenging task.

Because most toxicity tests are conducted on relatively common, easily cultured organisms, the effect of chemicals on more exotic species is relatively unknown. Some of these untested aquatic plants and animals may be ecological *keystone* or *habitat-forming* species, on whose survival the ecosystem rests (for example, kelp in near-shore marine waters often provides food and habitat for a wide variety of other species). Some untested species may be endangered and in need of special protection to ensure their survival. Future criteria may need to take these species into account.

One way special types of plant and animal communities may be protected is through site-specific criteria. These criteria are modifications of the original national criteria that are based on a nationwide data set. Site-specific criteria are based on a more limited data set pertaining only to those species potentially present in a particular water body. Site-specific criteria may be more stringent than national criteria if extremely sensitive species are present in the water body. On the other hand, site-specific criteria may be less stringent than national criteria if certain sensitive species cannot possibly be present in the water body (for example, warm-water streams of the midwestern U.S. could not naturally support trout, which are extremely sensitive to cyanide). Site-specific criteria may be used in the future to tailor water quality decisions to individual water bodies.

Finally, future criteria may be based on a better understanding of ecological risk associated with chemicals and other nonchemical parameters (for example, habitat-substrate properties, flow, and vegetative cover). At present, there is a limited understanding of how long plants and animals may be exposed to certain chemicals with no adverse effects. In some cases, aquatic communities may be exposed to high concentrations of chemicals for short periods (typical of spills, for example). Future criteria may incorporate more complete knowledge about how such exposures affect aquatic species. They may also incorporate knowledge about how often criteria may be exceeded without causing ecological damage.

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Chapter 3
Biomonitoring Test Procedures

20 Basis for Biomonitoring Tests
21 Types of Tests
23 Acute Tests
24 Chronic Tests
25 Reference Toxicity Tests
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30 Sample Procurement and Holding
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This chapter focuses on freshwater and marine chronic and acute toxicity tests used in the National Pollutant Discharge Elimination System (NPDES) permit program to identify effluent and receiving water containing toxic materials in acutely or chronically toxic concentrations. U.S. Environmental Protection Agency (U.S. EPA) toxicity testing manuals provide complete details on (1) types of tests, (2) health and safety, (3) quality assurance, (4) facilities and equipment, (5) test organisms, (6) dilution water, (7) sampling and sample handling, (8) test procedures, (9) data analysis, and (10) report preparation (U.S. EPA 1989a, 1989b, 1989c, 1991a, 1991b, 1992a, 1992b, 1993a, 1993b, 1993c, 1994a, 1994b, and 1994c). Here, types of tests, test organisms, and

general test procedures are described with references to appropriate U.S. EPA manuals. Certain states may have alternative procedures or reporting requirements, so it is important to carefully read the biomonitoring portion of the NPDES permit and communicate with the appropriate personnel in the state regulatory office to fully understand toxicity testing requirements. In addition to a description of toxicity testing for NPDES compliance, a brief description of mesocosm and sediment toxicity testing is included in this chapter. Many terms have been used over the years to describe toxicity tests performed for NPDES compliance: bioassays; toxicity tests; biomonitoring; and, most recently, whole effluent toxicity (WET) tests. In this chapter, the term *toxicity test* or *WET test* will be used to describe the type of biomonitoring applicable to NPDES compliance.

BASIS FOR BIOMONITORING TESTS

The goal of whole effluent toxicity testing is to simulate an effluent's effect on the receiving water body. It is important to note that an adverse (toxic) effect observed under laboratory conditions indicates only the *potential* for an adverse effect on the receiving water body. Although laboratory simulations (that is, toxicity tests) may be the most practical indicators of potential risk, they are not definitive or absolute for predicting actual effects. Certain types of pollution (for example, phosphorus) can cause stimulatory effects, including increased cellular growth rates within algae or other plant life. While the initial stimulatory effects may not appear to create a situation that is detrimental to the receiving water body, long-term effects of large algal blooms may lead to eutrophication or complete loss of aquatic life.

There are a number of factors that prohibit laboratory toxicity tests from being more absolute in their predictive power, including the inherent variability of biological systems. Organisms of identical characteristics, such as age and species, may respond differently when exposed to the same contaminant at the same concentration under identical conditions. Reduction of this variability factor and other causes of variability are addressed in the standard testing protocols provided in this chapter. To summarize, toxicity testing is an analytical tool used to predict the level of risk associated with wastewater discharge on a receiving water. As a tool used to estimate risk, it is useful.

There are typically good aquatic and sediment toxicology data available as "water and sediment quality criteria" or as "criteria for the protection of aquatic life" in the Regional Water Quality Control Board, U.S. EPA, and toxicological literature. Some local water and sediment quality criteria may have already been developed for a specific geographic range or body of water by local regulatory agencies, or such criteria may still have to be established using water and sediment toxicity tests and aquatic toxicology modeling techniques (Burton [Ed.], 1992, and Calabrese and Baldwin, 1993).

For more information on the testing protocols that follow, refer to the following U.S. EPA manuals: *Methods of Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (1993a), *Short-Term Methods of Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (1994a), and *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (1994b). Methods in these manuals are referenced in Table IA, 40 CFR Part 136, and are, therefore, approved methods for acute and chronic toxicity tests. Modifications of these tests are also used in toxicity reduction evaluations and toxicity identification evaluations to identify the toxic components of an effluent and aid in the development and implementation of toxicity reduction plans (U.S. EPA, 1989a, 1989b, 1989c, 1991a, 1992a, 1993b, 1993c). Although U.S. EPA manuals are typically referenced in NPDES permits, toxicity test methods for a variety of organisms are also available from other sources (APHA, 1995; ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.,* 1995). Guidance for the implementation of toxicity tests in the NPDES program is provided in *Technical Support Document for Water Quality-Based Toxics Control* (U.S. EPA, 1991b).

TYPES OF TESTS

The two significant categories of toxicity testing are acute tests and chronic tests. An acute test lasts 96 hours or fewer, and mortality is the measured endpoint. A chronic test is a long-term test in which sublethal effects, such as growth and reproduction, are typically measured in addition to mortality. U.S. EPA chronic tests required in NPDES permits have been shortened to 7 days by focusing on the most sensitive life-cycle stages; these tests are often referred to as short-term chronic tests. Table 3.1 summarizes short-term chronic and acute tests recommended by U.S. EPA for the NPDES permit. The type of test(s) required depends on NPDES permit requirements, objectives of the test, available resources, requirements of the test organisms, and effluent characteristics such as variability in flow or toxicity. The permit writer will determine the requirements for toxicity test(s) by considering such factors as (1) dilution, (2) effluent variability, (3) exposure variability, (4) species sensitivity, (5) type of industry or publicly owned treatment works, (6) existing data on toxic pollutants, and (7) type of receiving water and designated uses (U.S. EPA, 1991b). For example, U.S. EPA recommends that a discharger conduct acute toxicity testing if the dilution of the effluent is greater than 1 000:1 at the edge of the mixing zone and either acute or chronic toxicity testing if the dilution of the effluent falls between 100:1 and 1 000:1 at the edge of the mixing zone (U.S. EPA, 1991b).

Whether the required test is acute or chronic, the objective of the test is to estimate the "safe" or "no effect" concentration, which is defined as the concentration that will permit normal propagation of fish and other aquatic

Table 3.1 **Common acute and chronic toxicity test organisms and endpoints used for National Pollutant Discharge Elimination System permit compliance (U.S. EPA 1993a, 1993b, 1993c, 1994a, 1994b, and 1994c)**

Species/common name[a]	Test duration	Test endpoints
Freshwater, acute		
Ceriodaphnia dubia Cladoceran	24, 48, or 96 hours	Mortality
Daphnia pulex, D. magna Cladoceran	24, 48, or 96 hours	Mortality
Pimephales promelas Fathead minnow	24, 48, or 96 hours	Mortality
Oncorhynchus mykiss Rainbow trout	24, 48, or 96 hours	Mortality
Salvelinus fontinalis Brook trout	24, 48, or 96 hours	Mortality
Freshwater, chronic		
Ceriodaphnia dubia Cladoceran	Approximately 7 days (until 60% of control have three broods)	Survival, reproduction
Pimephales promelas Fathead minnow	7 days	Growth, survival
Pimephales promelas Fathead minnow	7–9 days	Embryo-larval survival, percent hatch, percent abnormality
Selenastrum capricornutum Freshwater alga	96 hours	Growth
Marine, acute		
Mysidopsis bahia Mysid shrimp	24, 48, or 96 hours	Mortality
Cyprinodon variegatus Sheepshead minnow	24, 48, or 96 hours	Mortality
Menidia beryllina, M. menidia, M. peninsulae Inland, Atlantic, and Tidewater silverside	24, 48, or 96 hours	Mortality
Marine, chronic		
Arbacia punctulata Sea urchin	1.5 hours	Fertilization

Table 3.1 Common acute and chronic toxicity test organisms and endpoints used for National Pollutant Discharge Elimination System permit compliance (U.S. EPA 1993a, 1993b, 1993c, 1994a, 1994b, and 1994c) (continued)

Species/common name[a]	Test duration	Test endpoints
Champia parvula Red macroalga	7–9 days	Fertilization (cystocarp production)
Mysidopsis bahia Mysid shrimp	7 days	Growth, survival, reproduction
Cyprinodon variegatus Sheepshead minnow	7 days	Growth, survival
Cyprinodon variegatus Sheepshead minnow	7–9 days	Embryo-larval survival, percent hatch, percent abnormality
Menidia beryllina Inland silverside	7 days	Growth, survival

[a] Supplemental species may include amphipods (*Hyalella* spp. and *Gammarus* spp.), mayflies (*Hexagenia* spp., *Baetis* spp., and *Empherella* spp.), midges (*Chironomus* spp.), stone flies (*Pteronarcys* spp.), and fish (*Cyprinella leedsi*, bannerfin shiner; *Lepomis macrochirus*, bluegill sunfish; and *Ictalurus punctatus*, channel catfish).

life in receiving water (U.S. EPA, 1994a). To conduct a WET test, an effluent sample is collected, diluted (typically with a laboratory control water, but receiving water may be used), and placed in test chambers with the chosen test species. Most toxicity tests follow one of the following basic designs: static test, in which the test solutions, or samples, are not changed for the duration of the test; static renewal test, in which each sample is changed all at once with a new sample, preferably every 24 hours, until the test ends; static renewal flow test, in which each sample is changed with a new daily sample by completely pumping each new sample through the test at a constant rate, preferably within a 24-hour period, for the duration of the test; or flow-through test, in which the sample consists of a continuous stream of test solution that flows through the test system at a constant rate during the test's duration (ASTM, 1993; Cairns and Niederlehner [Eds.], 1995; and Hoffman *et al.*, 1995).

ACUTE TESTS. The goal of the acute test is to determine if an effluent contains toxic materials in acutely toxic concentrations. Acute WET tests are typically designed so that the concentration of effluent that is lethal to 50% of the test organisms (LC_{50}) in a prescribed time period can be determined. Results of acute tests that use mortality as an endpoint may be used to design future toxicity testing protocols (including using chronic test procedures). Originally, acute tests were the "kill-and-count" type of tests that used species that were relatively easy to handle, were from older life stages ("heartier"), and required

a relatively short test duration. Thus, acute tests cost less in effort and money to conduct than chronic tests. Historically, acute tests were the criteria for regulatory determinations. Acute tests are being replaced by similar tests that provide chronic data using more sensitive life stages or species. Well-designed and well-analyzed acute tests are still used to provide lethal concentration data for use in particular regulatory criteria (Toxic Substances Control Act), manufacturers' specification data sheets, new product effects data, and NPDES permits, and in scientific research because such tests can be more cost effective. However, some acute tests are also used for screening purposes, as they are useful for deciding which types of chronic tests could be used and determining which sample locations are potentially toxic (ASTM, 1993; U.S. EPA, 1993a; and WEF, 1993).

The general procedures for running acute tests involve placing test organisms in test chambers that contain full-strength and/or diluted effluent that has been adjusted to the prescribed temperature. Acute tests may be static, static renewal, or flow-through. Flow-through testing often represents a better simulation of actual exposure conditions because organisms are continuously exposed to test solutions flowing in and out of test chambers for the duration of the test. Such testing is most often performed under laboratory conditions but may also be done in the field via suspension of caged organisms within the actual effluent flow stream (*in situ* testing). Flow-through testing is frequently used when physical and/or chemical characteristics of the test water fluctuate rapidly, such as high biochemical oxygen demand (BOD) loads that may deplete dissolved oxygen levels or waters that undergo significant fluctuations in pH.

Acute tests may be range-finding tests, screening tests, or definitive tests. Range-finding tests typically last only 24 hours and use a wide range of effluent concentrations. They are used to determine the highest concentration that does not kill test organisms and the lowest concentration that will kill all of the organisms. Screening tests typically involve testing with full-strength and/or one concentration of effluent. Endpoints of these acute tests are percent mortality. "Pass" or "fail" NPDES permit limits may be based on percent survival above a certain level. Definitive tests use various dilutions of full-strength effluent (typically 6.25, 12.5, 25, 50, and 100%) and last from 24 to 96 hours. The LC_{50} is then calculated and used to determine compliance with the NPDES permit.

CHRONIC TESTS. The goal of the chronic test is to determine if an effluent contains toxic materials in chronically toxic concentrations. In addition to mortality, chronic tests can help identify more specific long-term effects on test organisms such as reduced fertilization, reproduction, growth, teratogenicity (ability to cause developmental deformities), hatchability, and algal cell growth stimulation (Table 3.1). The endpoints of the chronic tests can be more

sensitive because younger test organisms often grow, develop, reproduce, and live out a significant portion of their lives in the particular test system.

The objective of most chronic tests is to determine the highest concentration of a series of effluent dilutions that will have no effect on organisms in the receiving water. This information can be used to predict whether normal dilution of the effluent by the receiving water will eliminate toxic effects that are seen in undiluted effluent. Because of multiple parameters monitored during chronic tests, more complicated statistical data analysis is required.

Effects that are monitored during chronic tests vary with the type of test. Endpoints from chronic toxicity tests include the no observed effect concentration (NOEC), lowest observed effective concentration, chronic value, maximum acceptable toxicant concentration, effective concentration, and inhibition concentration (IC). Definitions of these terms and their statistical derivations can be found in the chronic test data analysis section in Chapter 4 and in the Glossary.

REFERENCE TOXICITY TESTS. In addition to chemical and instrumental quality control, reference toxicant tests are run to determine the sensitivity of the batch of organisms being tested and to evaluate the test procedure. Test organisms are exposed to various concentrations of U.S. EPA-approved reference toxicants. These reference toxicants include sodium chloride, potassium chloride, cadmium chloride, copper sulfate, sodium dodecyl sulfate, and potassium dichromate. The degree of each organism's response to various concentrations of the reference toxicant is measured and compared to acceptable ranges. If organisms respond outside of acceptable ranges, test organisms are considered either too sensitive or not sensitive enough to be used in the toxicity tests. For each type of test, there may be other criteria that must be met for the test to be considered valid.

TEST ORGANISMS

The type of test and the species to be used are specified in the NPDES permit and are typically those listed in Table 3.1. Many other species have been and can be used for toxicity testing in applications outside of NPDES permits; some of these species are listed in Tables 3.2 and 3.3. The most common freshwater test organisms are the fathead minnow (*Pimephales promelas*, shown in Figure 3.1), cladocerans or water fleas (*Ceriodaphnia dubia,* shown in Figure 3.2, and *Daphnia magna*), and freshwater alga (*Selenastrum capricornutum*). The most common marine test organisms are the sheepshead minnow (*Cyprinodon variegatus,* shown in Figure 3.1), inland silverside (*Menidia beryllina,* shown in Figure 3.1), mysid shrimp (*Mysidopsis bahia*, shown in Figure 3.2), and marine alga (*Champia parvula*). Alternate species may be used for NPDES compliance after petitioning U.S. EPA and providing supporting data regarding tests performed on alternate species. For the

biomonitoring approach to be valid and defensible, toxicity tests that are used must encompass a broad taxonomic range of organisms so as to best represent actual receptor (resident) organisms that would be subject to potential effects from waste streams or human activities. Organisms chosen for testing, or indicator species, serve to represent the main food web pathways associated with the main trophic levels. The indicator species that are tested also have to represent, ecologically and taxonomically, those major taxonomic groups or foraging guilds of receptor organisms that would actually be located within the area of concern (the affected environment); however, such indicator species themselves need not live within the area of concern for their biomonitoring data to be useful. Consequently, there are occasions where actual receptor organisms are numerous enough and cited adequately in the ecotoxicological literature such that these particular target receptors are also commonly used toxicity test organisms.

Table 3.2 Standard acute and chronic toxicity bioassays with "liquid media" for marine and estuarine species (ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.*, 1995)

Liquid-media acute bioassays (mortality: 48-h and/or 96-h) (note: longer tests may require renewal or flow-through protocols, rather than static)	
Bacteria (luminescence; 5, 15, 30 min.):	
Photobacterium phosphorum	(Microtox)
Zooplankton:	
Arcartia spp.	Copepods
Leuresthes tenuis	Grunion (embryos)
Algae (2-day/7-day reproduction):	
*Champia parvula**	Red algae (cystocarps)
Amphipods:	
Ampelisca adbita; Corophium insidiosum	Amphipods
Eohaustorius estuarius; Grandidierella japonica	Amphipods
Rhepoxynius abronius	Amphipods
Polychaetes:	
Capitella capitata; Hediste (Nereis) spp.	Polychaete worms
Neanthes arenaceodentata	Polychaete worm
Mysids:	
*Holmesimysis costata**	Mysid shrimp
Mysidopsis spp., *Neomysis* spp.*	Mysid shrimp

Table 3.2 **Standard acute and chronic toxicity bioassays with "liquid media" for marine and estuarine species (ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.*, 1995) (continued)**

Shrimp:	
Crangon spp.	Bay shrimp; sand shrimp
Palaemonetes spp.; *Pandalus* spp.; *Penaeus* spp.	Grass shrimp; shrimp
Sicyonia ingentis	Ridgeback prawn
Pelecypods:	
Crassostrea spp.; *Ostrea* spp.*	Pacific oyster; eastern oyster; oysters
*Mytilus edulis**	Blue mussel
Macoma nasuta; Protothaca staminea	Bentnose clam; littleneck clam
Crabs:	
Callinectes sapidus; Carcinus maenas	Blue crab; green crab
Hemigrapsus spp.; *Pachygrapsus* spp.	Shore crabs
Uca spp.	Fiddler crab
Fish:	
*Atherinops affinis**	Top smelt
Citharichthys stigmaeus	Speckled sanddab
Clevelanda ios	Arrow goby
Clupea harengus	Herring
Cymatogaster aggregata	Shiner perch
*Cyprinodon variegatus**	Sheepshead minnow
Fundulus heteroclitus	Mummichog
Fundulus similis	Longnose killifish
Gasterosteus aculeatus	Threespine stickleback
Lagodon rhomboides	Pinfish
Leiostomus xanthurus	Spot
Menidia spp.*	Silversides
Oligocottus maculosus	Tidepool sculpin
Paralichthys spp.	Flounder
Parophrys vetulus	English sole
Platichthys stellatus	Starry flounder

Table 3.2 Standard acute and chronic toxicity bioassays with "liquid media" for marine and estuarine species (ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.*, 1995) (continued)

Liquid-media chronic bioassays (mortality, growth, reproduction; 2-h, 48-h, 96-h, and 7-day) (most tests deal with larval stages or development; mostly daily renewal or flow-through tests)	
Mysids; pelecypods; and fish (above *)	
Plankton (96-h diatom growth test):	
Skellotenemma spp.; *Thalassiosira* spp.	
Red algae reproduction test (above *):	
Champia parvula	
Echinoderms:	
Arbacia punctulata; Lytechinus pictus	Sea urchins
Strongylocentrotus spp.	Green sea urchin; purple sea urchin
Dendraster excentricus	Sand dollar

U.S. EPA recommends that a minimum of three species representing three different phyla (for example, a fish, an invertebrate, and a plant) (U.S. EPA, 1991b) be used for WET tests in NPDES permits. However, typically only two species are required although this will depend on how well the effluent has been characterized, the available receiving water dilution, use classification and existing uses of the receiving water, and other considerations (U.S. EPA, 1991b). Approved organisms for toxicity tests should be cultured or purchased from a commercial supplier. Collecting organisms from the receiving water is typically not recommended (U.S. EPA, 1994a and 1994b).

Almost all wastewater generated in inland areas is discharged to freshwater streams and lakes. The biology of plants and animals living in fresh water, where salt concentrations are low, is often different from that of marine life. Therefore, toxicity tests of effluents discharged in these areas must use freshwater life forms as the target organisms when determining whether toxic substances are present. Freshwater test species are typically used when the receiving body of water contains less than 1 part per thousand (ppt) salinity, regardless of the salinity of the effluent. Receiving waters are considered saline if they contain greater than 5 ppt salinity. Where the salinity of the receiving water is greater than or equal to 1 ppt, the choice of organism depends on state water quality standards and/or permit requirements. Exceptions to this guidance include Florida (U.S.), where marine species must be used when *effluent* salinity is greater than 1 ppt.

Table 3.3 **Standard marine and estuarine sediment toxicity tests and species (ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.*, 1995)**

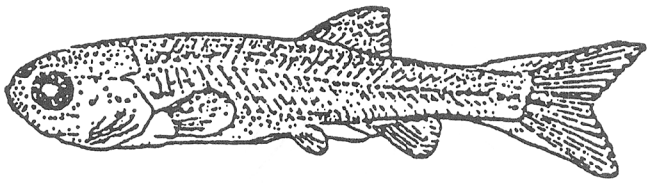
Whole sediments	
Amphipods (10-day mortality and/or reburial tests):	
Ampelisca adbita	Amphipod
Corophium insidiosum	Amphipod
Eohaustorius estuarius	Amphipod
Grandidierella japonica	Amphipod
Rhepoxynius abronius	Amphipod
Polychaetes (96-h to 10-day mortality and/or juvenile growth tests):	
Capitella capitata	Polychaete worm
Hediste (Nereis) spp.	Polychaete worm
Neanthes aeraceodentata	Polychaete worm
Shrimp (96-h to 28-day mortality and/or bioaccumulation tests):	
Sicyonia ingentis	Ridgeback prawn
Pelecypods (96-h to 28-day mortality and/or bioaccumulation tests):	
Macoma nasuta	Bentnose clam
Protothaca staminea	Littleneck clam
Fish (96-h to 28-day mortality and/or bioaccumulation tests):	
Clevelanda ios	Arrow goby
Liquid-phase sediments	
Algae (2-day/7-day reproduction test):	
*Champia parvula**	Red algae
Bivalve larvae (48-h mortality and growth tests):	
Crassostrea gigas	Pacific oyster
Crassostrea virginica	Eastern oyster
Mytilus edulis	Blue mussel
Ostrea spp.	Oyster
Echinoderm embryos (2-h, 48-h, or 96-h mortality and growth tests):	
Arbacia punctulata	Sea urchin
Dendraster excentricus	Sand dollar
Lytechinus pictus	Sea urchin
Strongylocentrotus droebachiensis	Green sea urchin
Strongylocentrotus franciscanus	Sea urchin
Stronglyocentrotus purpuratus	Purple sea urchin

Table 3.3 Standard marine and estuarine sediment toxicity tests and species (ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.*, 1995) (continued)

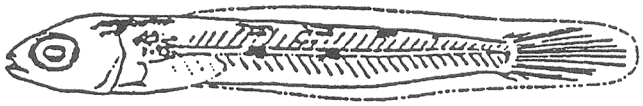
Zooplankton (48-h mortality tests):	
Arcartia spp.	Copepods
Leuresthes tenuis	Grunion (embryos)
Mysids (96-h mortality tests):	
Holmesimysis costata	
Mysidopsis spp.	
Neomysis spp.	
Fish (96-h mortality tests):	
Atherinops affinis	Top smelt
Citharichthys stigmaeus	Speckled sanddab
Cymatogaster aggregata	Shiner perch
Menidia spp.	Silversides
Liquid-phase/sediment extracts	
Red algae 2-day/7-day reproduction test (above*):	
Organic solvent extract:	
48-h anaphase aberration test:	
Oncorhynchus mykiss	Rainbow trout (formerly *Salmo gairdnerii*)
Microtox luminescence test (5, 15, 30 minutes):	
Photobacterium phosphorum	
Saline extract:	
Microtox luminescence test (15 minutes):	
Photobacterium phosphorum	

SAMPLE PROCUREMENT AND HOLDING

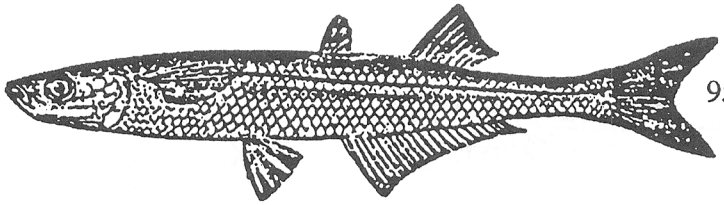
The sample collection site and type of sample (grab or composite) is specified in the NPDES permit and may not be the same type collected for other compliance data (for example, metals). Any changes in the sample location, whether temporary or permanent, must have the approval of the regulating agency. Proper sample collection and handling is an essential aspect of all toxicity tests. The goal of the sample collection process is to obtain a sample that reflects the water quality as closely as possible. According to U.S. EPA (1991b), "The use of a grab sample or a composite sample is based upon the



Pimephales promelas
(Fathead minnow) larvae



Cyprinodon variegatus
(Sheepshead minnow) larvae

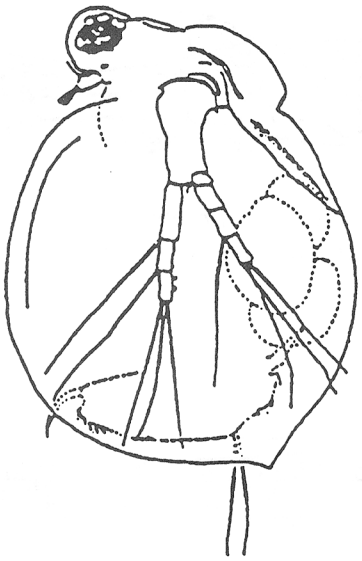


Menidia beryllina
(Inland silverside) adult

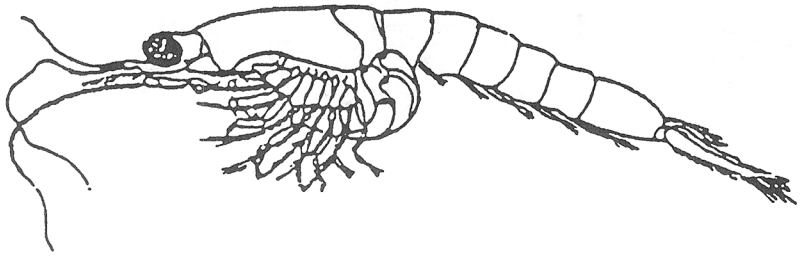
Figure 3.1 **Three fish commonly used for toxicity tests: fathead minnows are used for freshwater toxicity tests; sheepshead minnow and inland silverside are used for marine toxicity tests (modified from U.S. EPA [1993a])**

objectives of the test and an understanding of the long-term operations and schedules of the discharger."

When composite samples are required, they are ideally collected using refrigerated samplers capable of collecting flow-paced, 24-hour samples. When automation is not possible, grab samples taken at representative times



Ceriodaphnia dubia



Mysidopsis bahia

Figure 3.2 **Two invertebrates commonly used for toxicity tests: *Ceriodaphnia dubia* are used for freshwater toxicity tests, with adult length of approximately 900 μm; *Mysidopsis bahia* are used for marine toxicity tests, with adult length of approximately 4 to 10 mm (modified from U.S. EPA [1993a])**

can be used. For field use, portable samplers that can be filled with ice to maintain an approximate temperature of 4°C are also acceptable. The total volume of sample collected will depend on the test volume and the number of

replicates for each required test; several extra litres should be collected for repeat testing and/or further analysis to identify toxicants.

Chemicals in the effluent sample may degrade during collection or holding. Both artifactual (false-positive) and false-negative results can occur if sample collection is performed incorrectly. Examples of causes of false positives caused by sampling include improperly cleaned sample containers, containers for storage methods that permit the unintentional introduction of toxicants, or unclean sample compositors. Both chemical and biological cleanliness are important. If bacterial or viral contamination is introduced, it can weaken or kill test organisms. Biological contamination can be difficult to detect.

Initially, sample collection containers should be new, and subsequently they should be used exclusively for toxicity tests. Containers should be cleaned before use with mild "ultrapure" hydrochloric or nitric acid. They should then be rinsed thoroughly with ultrapure 18-M-ohm water. If manual collection is performed, the containers should be rinsed with a small amount of the effluent sample just before collection of the first aliquot.

False-negative results (a toxic sample losing its toxicity before testing) can be caused by improper sample collection temperatures, excessive holding times, improper choice of sample container material (glass can absorb metals and plastics can absorb pesticides), or excessive shaking of the sample, causing loss of volatile chemicals.

Special compositors are available for the collection of samples when volatile organic chemicals are suspected. The choice of sample container material can vary depending on what type of toxicant is expected. Typically, sample containers made of glass are preferable, except in cases of suspected heavy metals, for which polyurethane or polyethylene plastics are appropriate. Stainless steel containers are also acceptable for general use.

Tests must be initiated within 36 hours of collection or removal of the sample from the compositor. Samples must be maintained at 4°C during collection, shipping, and holding during the test. Sample preservatives should never be used.

Toxicity tests may be conducted at the discharging facility or at a qualified contract laboratory. Many states have biomonitoring laboratory certification programs, but there is not a national certification program. Contract laboratories are required to provide information to their clients regarding their performance on quality assurance samples, such as the discharge monitoring report quality assurance program sponsored by U.S. EPA. Chapter 4 discusses quality assurance/quality control procedures used during testing and for laboratory certification. Advantages to conducting tests in house include better scheduling of tests, minimizing the degradation over time of unstable or volatile toxicants, and the ability to repeat tests or run modified versions of test protocols for research purposes. Cost comparison of in-house versus contract laboratory testing is also an important consideration.

TEST PROCEDURES

When performing WET tests for NPDES compliance, appropriate U.S. EPA manuals previously cited in this chapter provide complete details of each test procedure. Some of the procedures common to all of these toxicity tests are provided below. Toxicity tests conducted for other purposes (for example, research and development) may use alternative but, in many cases, similar procedures found elsewhere (for example, APHA, 1995; ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.*, 1995).

TEST SETUP. Aliquots of samples are removed from refrigerated holding and allowed to slowly warm to the test temperature. All samples, controls, and reference toxicant test solutions must be at correct test temperatures before initiating the test and when renewing solutions. The remainder of the sample must be held at 4°C. Sample temperature, dissolved oxygen, pH, and chlorine are recorded before adding test organisms. Throughout testing procedures, the temperature must be maintained within ±1°C of the test protocol.

Lighting must be cyclical, with 16 hours of light daily. Light intensity should be 538 to 1 076 lx (50 to 100 ft-c), which is approximately ambient laboratory light level.

At the end of each 24-hour period, dissolved oxygen, pH, and temperature of test solutions are recorded. Each time solutions are renewed, the same measurements should be recorded. Additional monitoring of conductivity, alkalinity, hardness, and ammonia are also required in test protocols. Small amounts of chlorine can be toxic. Chlorine can be removed from the sample before testing only if specified in the NPDES permit. Sodium sulfite or sodium thiosulfate can be used for chlorine removal.

AMMONIA TOXICITY. Ammonia toxicity is common and can cause severe problems in biomonitoring tests. The topic of ammonia toxicity is introduced here in the early part of the test procedures section because the possible complications that ammonia toxicity can present should be considered early in the development of a toxicity testing plan. Ammonia toxicity is found primarily in wastewater effluent but can occur in any water that contains significant ammonia and can mask the effects of other toxicants. To be sure that toxicants other than ammonia are not present, ammonia toxicity must first be identified. Vertebrates, especially fish, are more sensitive to ammonia than invertebrates. This species sensitivity difference, with knowledge of ammonia concentrations and the pH of the sample, can help identify ammonia toxicity. If high levels of total ammonia (more than 5 mg/L) are expected, considerations for ammonia toxicity should be made. Ammonia toxicity is directly related to the pH of the test solution. The un-ionized ammonia (NH_3) portion of the total ammonia is the toxic portion. At higher pH, the percent of un-ionized ammonia increases. Some of the solutions to the ammonia

toxicity problem include maintaining a low pH with the use of carbon dioxide in a closed environment, removing the ammonia by treating the sample with zeolite, or removing the ammonia by air stripping at high pH. One drawback of using the zeolite method is that zeolite will also bind other positively charged molecules, especially heavy metals, leading to the potential for false-negative results. Air stripping at high pH can degrade sensitive toxicant and remove volatile compounds. Special manipulation of the samples for alleviation of ammonia problems requires approval from the regulating agency.

OXYGEN DEPLETION. Samples containing BOD will cause a rapid depletion of oxygen during the test. Oxygen depletion will stress the organism and can cause false-positive results. The BOD or chemical oxygen demand tests can be used to predict whether oxygen depletion will occur during testing. If the dissolved oxygen concentration falls below 4 mg/L for warm water species and 6 mg/L for cold water species, aeration must be initiated. Aeration should be gentle (100 bubbles per minute). If any samples require aeration, all test chambers should be aerated. Aeration will cause the pH to increase and can complicate problems caused by un-ionized ammonia. Once the temperature has been checked and the above items have been addressed, organisms can be added to test solutions.

SAMPLE FILTRATION. Excessive filtration of effluent can remove toxicants that are bound to large particulate matter. Coarse filtration of the sample through 1- to 4-mm mesh screens is acceptable for removing large particles. Microorganisms that are pathogenic to the test organism can be a source of false-positive tests. Waters from wastewater lagoons may contain zooplankton, which can interfere with test results by providing additional food for fish or by competing with daphnids used in the tests. In these samples, filtration through a 60-µm pore size filter is recommended. Surface water used in algal growth tests should also be filtered through a 0.45-µm pore size filter before use.

DILUTION WATER. The type of water that is to be used for diluting samples is specified in the NPDES permit. Sources of dilution water include synthetic, receiving water, or other nontoxic surface water (marine or fresh). If the objective of the test is to estimate the acute toxicity of the effluent, synthetic dilution water is used. If the objective of the test is to estimate the acute toxicity of the effluent in uncontaminated receiving water, synthetic water, receiving water, or nontoxic groundwater may be used. For tests with objectives of determining the additive effects of the discharge on already contaminated surface water, receiving water collected upstream of the outfall is used for dilution. Tables 3.4 and 3.5 provide formulas for various freshwater synthetic dilution waters. Formulas for synthetic saltwater can be found in U.S. EPA publications (1993a and 1994b).

Table 3.4 Preparation of synthetic freshwater using mineral water (U.S. EPA, 1993a)

Water type	Volume of mineral water added, mL/L[a]	Proportion of mineral water, %	Final water quality		
			pH[b]	Hardness[c]	Alkalinity[c]
Very soft	50	2.5	7.2–8.1	10–13	10–13
Soft	100	10.0	7.9–8.3	40–48	30–35
Moderately hard	200	20.0	7.9–8.3	80–100	60–70
Hard	400	40.0	7.9–8.3	160–180	110–120
Very hard[d]	—	—	—	—	—

[a] Add mineral water to ultrapure (well-distilled) water or equivalent to prepare diluted mineral water.
[b] Approximate equilibrium pH after 24 h of aeration.
[c] Expressed as mg $CaCO_3$/L.
[d] Dilutions of mineral water form a precipitate when concentrations equivalent to "very hard water" are aerated.

Table 3.5 Preparation of synthetic freshwater using reagent-grade chemicals (U.S. EPA, 1993a)

Water type	Reagent added, mg/L[a]				Final water quality		
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl	pH[b]	Hardness[c]	Alkalinity[c]
Very soft	12.0	7.5	7.5	0.5	6.4–6.8	10–13	10–13
Soft	48.0	30.0	30.0	2.0	7.2–7.6	40–48	30–35
Moderately hard	96.0	60.0	60.0	4.0	7.4–7.8	80–100	60–70
Hard	192.0	120.0	120.0	8.0	7.6–8.0	160–180	110–120
Very hard	384.0	240.0	240.0	16.0	8.0–8.4	280–320	225–245

[a] Add reagent-grade chemicals to deionized water.
[b] Approximate equilibrium pH after 24 h of aeration.
[c] Expressed as mg $CaCO_3$/L.

Synthetic water is made from ultrapurified water (18 M-ohm) to which reagent-grade chemicals are added to the appropriate hardness or salinity. Synthetic freshwater may also be prepared using ultrapurified water mixed with commercially available mineral water, and synthetic seawater may be prepared by adding commercial sea salts. Dilution waters for freshwater tests are classified by their hardness and range in hardness from very soft (10 to 13 mg/L calcium carbonate [$CaCO_3$] to very hard (280 to 320 mg/L $CaCO_3$). The hardness or salinity of synthetic dilution water should mimic the receiving water. Chemical parameters of nonsynthetic dilution water should also be similar to the effluent being tested. Dilution water should not be toxic. If reduced

survival occurs in control animals, the water should be discarded, and the test should be reinitiated with a fresh supply or an alternative source should be used.

ORGANISM LOADING. Care must be taken when adding organisms to test solutions so that they are not injured during the transfer from holding containers or tanks to the test chambers. When adding test organisms, a minimal volume of holding water should be transferred. Excessive addition of holding water that contains organisms will dilute samples, thereby decreasing the concentration of constituents in the test solution. Test chamber size and sample volumes are specified for each test (U.S. EPA, 1993a, 1994a, and 1994b).

Overcrowding of organisms in test chambers can cause added stress to test organisms. Organisms should be randomly placed in the test chamber to ensure minimal bias caused by differences in animal care and stress during holding. Tests are run in duplicate with equal numbers of test organisms in each chamber. Replicate test chambers should respond similarly. If animals in the replicate test chambers do not respond to the effluent in the same manner, glassware cleanliness or other differential adverse treatment should be investigated.

FEEDING DURING TESTING. Test organisms are fed during most of the tests. In acute tests, this is typically performed 2 hours before test solution renewal. Research has shown that certain toxic effects can be either reduced or enhanced by foods used during biomonitoring tests (for example, Winner, 1989). Specific foods and amounts to be added are described in the U.S. EPA manuals (1993a, 1994a, and 1994b).

MONITORING TESTS AND RECORDING DATA. As much data as possible on animals' responses to the effluent should be gathered. High-quality data recording is necessary so that data reviewed later will present an accurate picture of test results. Instruments used for monitoring should be calibrated and documented using normal quality control procedures. It is important to monitor closely the health and behavior of organisms during testing, such as surfacing, erratic swimming, or other visible signs of stress. This will enable the analyst to identify effects that may be related to the water quality. Bench sheets should be designed so that each test chamber's biological and chemical data can be recorded. Temperature, dissolved oxygen, and pH should be measured while animals are in test solutions rather than removing an aliquot and taking the measurement. The date and time of each monitoring as well as dates and times of samples that can be run at a later date (conductivity, alkalinity, hardness, and ammonia) should also be recorded. Bench sheets should be complete enough to be used as legal documentation.

ADDITIONAL METHODS FOR ASSESSING TOXICITY

MESOCOSM BIOMONITORING TESTS. The development of mesocosm bioassay testing protocols has been relatively recent. This type of test was classically referred to as an *aquatic field test* and has largely been associated with pesticide or new-product testing and registration (Graney *et al.*, 1994, and U.S. EPA, 1992b). A mesocosm is a multispecies, controlled ecosystem originally designed to address potential ecological effects from anthropogenic sources and to take into account between-species effects that would not be determined using bioassay tests on separate species. This falls into place with various types of ecological risk assessments in the area of "field validation tests" (or studies), which may be needed to address uncertainty associated with extrapolating single-species data to effects at a higher, "real-world" level. Two basic types of mesocosm studies exist: natural and simulated (Graney *et al.*, 1994). Natural mesocosm studies are not new; this is simply another way of interpreting or naming "field studies." Such field/mesocosm studies are those studies in which both the test system and exposure to the stressor are naturally derived. Such studies are site specific and are typically designed to evaluate the effects of chemicals from identified sources on a specific "natural" mesocosm because typically the results are only valid for that area. The simulated mesocosm is used more frequently. In a simulated mesocosm, a definite degree of control is placed over a natural area, or habitat is built or created. This ideology applies to large constructed pond systems; outdoor fabricated tanks or similar aquaculture systems; "limnocorrals" or "littoral enclosures," which are artificial enclosures either within the pelagic (open water) regions or enclosing the intertidal/littoral zones of water bodies; and "lenthic" or artificial stream systems. In the area of water pollution control, assessment, and treatment, such a concept actually exists in many wastewater treatment lagoons that are either natural or human-made (WEF, 1992 and 1993). The larger scope of mesocosm testing is probably beyond day-to-day biomonitoring studies except for when such testing is part of ecological risk assessments (Graney *et al.*, 1994, and U.S. EPA, 1989a and 1992b).

RESEARCH ON NEW FRESHWATER TOXICITY TEST METHODS. In addition to the acute and chronic tests outlined above, new methods are continuously being tested to provide accurate and reproducible aquatic toxicity information. Many new methods are designed to provide more rapid, less expensive toxicity information. Although not yet suitable for most regulatory purposes, these rapid assessment tests are potentially valuable for screening toxicity in large numbers of samples or providing less costly ways to help identify toxicants. Several methods have been tested extensively and are

commercially available in kit form (most of these tests require at least some technical experience with biological systems):

- A 15-minute test with bacteria to screen for the presence of metals (Bitton *et al.*, 1992);
- A 60-minute test to determine whether chemicals are present that can inhibit nitrifying bacteria (Stevens, 1988);
- A 60-minute test using fluorescent dye to determine acute toxicity to *Daphnia magna* (Janssen and Persoone, 1993); and
- A 2-day life-cycle test using rotifers to determine chronic toxicity (Snell and Moffat, 1992).

Several additional tests are still being developed but show promise:

- A mitochondrial respiratory response bioassay (Shannon *et al.*, 1991),
- An assay of stress proteins in fish tissue to determine the presence of potentially harmful metals concentrations (Janssen and Persoone, 1993), and
- Short-term tests with fish that use a variety of behavioral responses (such as erratic swimming) to measure the presence of toxicants (Little and Henry [Eds.], 1990).

In addition, several other recent tests have been developed to screen for the presence of teratogenic chemicals in water. These tests are less expensive than tests with standard laboratory animals such as white rats, but their application to terrestrial vertebrates (including man) is still uncertain. Among these recent tests are

- Frog embryo teratogenesis assay—this is a 96-hour test and is standardized by ASTM. It is also being used to determine whether toxicants are contributing to a perceived disappearance of sensitive amphibians (Waters *et al.*, 1982).
- Thirty-day tests using the fish *Oryzias latipes* (Japanese medaka)—this test requires the expertise of a biologist capable of discerning tumor formation in tissues that are slide mounted for microscopic examination (Bunton, 1991).

As described earlier, field and laboratory tests using freshwater microcosms or mesocosms are gaining wider use in toxicity analysis. These tests are intended to provide a community-level, rather than a single-organism-level, response to toxicants. The design of microcosm and mesocosm tests can be variable (communities may consist of protozoa only or may include organization levels from single-cell bacteria to vertebrate animals). Such tests are used primarily at the research level, but mesocosm data are now required for pesticide registration in the U.S.

SEDIMENT BIOMONITORING TESTS. The other form of acute or chronic estuarine and marine tests is the sediment toxicity test. These tests are divided into two categories, based on differences between the test media: liquid-phase tests and solid-phase, or whole-sediment, tests (U.S. EPA and U.S. ACOE, 1991, and U.S. EPA, 1994c). The liquid-phase sediment toxicity tests are similar to the liquid-media tests described previously, except that the liquid-phase test medium is an actual liquid fraction or "extract" made from mixing sediments, sludges, soils, or biosolids with any commonly used liquid media. The solid-phase sediment tests are designed to provide toxicity data on organisms that live in the sediment or are "closely associated" with sediments (ASTM, 1993; Burton [Ed.], 1992; and U.S. EPA 1994c). In the solid-phase sediment toxicity test, treatments consist of either the contaminated-sediment sample itself or a series of "percent-sediment concentrations," which are derived by combining "clean" sediments (control, or reference, sediments) with contaminated sediments according to test-specific methods (U.S. EPA and U.S. ACOE, 1991). Typical freshwater sediment test organisms include an amphipod (*Hyalella azteca*), midge (*Chironomus tentans*), and a worm (*Lumbriculus variegatus*) (U.S. EPA, 1994c).

Some typical acute liquid-phase sediment toxicity tests, which use liquid extract, also include 48-hour static algae or invertebrate tests (red algae, copepods, amphipods, mysids, shrimps, polychaete, or bivalves) and 96-hour static estuarine and marine fish tests (such as threespine sticklebacks, silversides, sheepshead minnow, and sanddabs) (ASTM, 1993; Burton [Ed.], 1992; and U.S. EPA, 1994b). Typical chronic liquid-phase sediment toxicity tests include the 2-day/7-day red algae (*Champia parvula*) reproduction test; the 48-hour bivalve oyster embryo survival and development test; the sea urchin embryo development test; the 7-day static-renewal invertebrate (mysid) survival, growth, and reproduction test; the 7-day static-renewal larval fish (silverside) survival and growth test; and the 96-hour static algal diatom (*Skeletonemma* spp. or *Thalassiosira* spp.) growth test (Burton [Ed.], 1992; Hoffman *et al.*, 1995; and U.S. EPA, 1994b).

Some typical acute and chronic solid-phase sediment toxicity tests include the 10-day amphipod survival and reburial test, the polychaete 96-hour (or 20-day) survival and growth test, the shrimp 96-hour (or 20-day) survival and growth test, the shrimp 96-hour bioaccumulation test, and the 96-hour arrow goby survival test (ASTM, 1993, and Burton [Ed.], 1992). A list of standard marine and estuarine sediment toxicity tests and species, including both liquid- and solid-phase tests, are shown in Tables 3.2 and 3.3 (ASTM, 1993; Burton [Ed.], 1992; and Hoffman *et al.*, 1995).

APPLICATION OF TEST RESULTS

USE OF FRESHWATER TOXICITY DATA. Application in Regulatory Permits. Many government agencies worldwide have begun incorporating toxicity testing or biomonitoring requirements to regulatory permitting processes. The goal of this process is to detect and eliminate toxicity from the aquatic environment. Biomonitoring techniques have been applied to both point and nonpoint sources of pollution. Although details for the permit process vary among jurisdictions, permit requirements typically include the following elements:

- Which species should be used as test organisms? Two or more species are typically required, representing a vertebrate, an invertebrate, and/or a plant species. A variety of test animals is desirable because no single organism is sensitive to all potential toxicants.
- Should tests be acute or chronic? Acute tests are typically applied where receiving stream dilutions are large or short-term exposures, such as from stormwater runoff. Chronic tests are typically applied in effluent-dominated situations.
- How frequently should tests be performed? Single testing events are applied where the probability of toxicity is low (for example, small municipalities or nonprocess industrial water). Frequent or continuous testing is applied where the probability of toxicity is high (for example, process water of certain industries or leachate from hazardous waste sites).
- What types of samples should be tested? Typically, 24-hour composite samples are required to detect potential pollutant spikes occurring during the course of a day. Stormwater tests may require only a "first flush" sample, which is most likely to contain potentially harmful pollutants.
- What constitutes significant toxicity? Often, no end-of-the-pipe acute toxicity is allowed (LC_{50} of less than 100% effluent) unless instantaneous mixing can be demonstrated. Allowable NOEC or IC_{25} values for chronic toxicity often depend on available upstream dilution. Many U.S. states allow a mixing zone of 25 to 50% of the receiving stream.
- Are there site-unique requirements? For water bodies in which multiple toxic discharges are suspected, regulatory agencies may take into account potential toxicity interactions. Waste load allocations for each discharger are sometimes required.

Application in Water Quality Criteria. A wise decision-making process in water pollution control requires a knowledge of what levels of a given chemical are likely to cause adverse effects on the aquatic environment. Most water quality criteria used in this process have been developed by assembling all

available toxicity test information, sorting out tests performed under similar conditions with good quality control, and finding a "safe" level that will protect most of the species potentially exposed to the chemical. Toxicity test data have played an important role in this process.

Criteria have been developed using well-defined, chemically simple dilution water because more reproducible test results are obtained. However, site-specific factors in more complex receiving waters (for example, higher organic carbon or complex buffer systems) may alter the actual toxicity of chemicals at these sites. Site-specific factors are probably more important in freshwater systems than in marine or estuarine waters because freshwater characteristics are more highly variable. Toxicity test data are increasingly important in developing site-specific criteria for use in the decision-making process.

USES OF MARINE/ESTUARINE TOXICITY DATA. There are four basic uses of estuarine and marine toxicity data, and these are related to the main reasons for performing all aquatic toxicity tests, as presented earlier:

- Testing can provide ecologically sound data for establishing or supporting water and sediment criteria;
- Toxicity data can serve to meet regulatory requirements;
- Data are good for analyzing the potential toxicity of unknown or complex mixtures; and
- Toxicity data can be used for determining toxic zones within the areas of concern, as part of an ecological risk assessment.

Toxicity test data can be compared to previously derived criteria or compared to similar work done by other researchers as part of a quality assurance/quality control review or ecological risk assessment to see if newer data are helpful. Recent data on ecological and toxicological effects can be used as regulatory criteria if no better data are available.

Toxicity data for meeting regulatory requirements can allow the reduction of current testing or study levels, or such new data can stimulate the need for more detailed research. The initiation of a toxicity reduction evaluation/toxicity investigation evaluation (TRE/TIE) is one such process that relies on updated toxicity test data. The TRE/TIE protocols (U.S. EPA, 1989b, 1989c, 1991a, 1993b, and 1993c) use aquatic bioassay tests to identify the sources or causes of toxicity and reduce or eliminate such effects. Various aquatic toxicity components of the NPDES program also use similar testing methods.

Estuarine and marine toxicity tests, like other aquatic toxicity tests, are good for analyzing the potential toxicity of unknown or complex mixtures. Testing of such complex mixtures is important for screening specific sites or areas for potential toxicity. Such toxicity tests are less costly and more ecologically relevant than an expensive array of chemical analyses, although

such chemical contaminant data are also needed before any toxicity correlations or potential etiologies can be made.

The expanding field of ecotoxicology and ecological risk assessment can use such data in a variety of ways. Aquatic toxicity data can be used for determining toxic areas in these environments. Such new data can be compared with water or sediment quality criteria or can be used for establishing regulatory criteria. The classic use of estuarine and marine (as well as freshwater) toxicity data in ecological risk assessments is for creating hazard quotients or toxicity units, which can be used with chemical concentration data for predicting and assessing the relative ecological risk of potentially toxic chemicals to target receptors or indicator species from various exposure pathways. Such site-specific toxicity data can be combined with similar chemical data to correlate site-specific chemical contamination with known levels of toxicity. Such new data can also be used to guide remedial activities, evaluate their effectiveness, and document effects on the local environment (U.S. EPA, 1992b). Such tests are also useful for establishing or confirming appropriate safety factors during later stages of an ecological risk assessment.

Estuarine and marine toxicity tests also form a vital component of what is known as the *triad* or *integrated assessment* approach to ecological risk assessments (Burton [Ed.], 1992). When such site-specific toxicity data and chemical contamination data are combined with other ecological assessment data, a triad, or *multicomponent array*, of information is formed. This concept was first developed by Chapman (1986) for deriving sediment quality criteria. This approach analyzes groups of different data sets, or *components*, collectively as either 3-, 4-, or 5-component integrated assessments. Individual components consist of relevant data sets of surface media chemistry, receptor/indicator species pathology or tissue chemistry, ecological community structure, and estuarine or marine toxicity data sets. The combination of these single classes of data into one of the above multicomponent arrays yields a better estimate of what the causes may be for any adverse effects within the estuarine and marine environments. This "weight-of-evidence" ideology is invaluable in the area of environmental quality regulation because it is frequently difficult to prove which anthropogenic sources are contributing to specific toxic effects seen in affected environments. Such weight-of-evidence documentation can be enough to warrant fines and penalties on responsible parties (U.S. EPA, 1989a and 1992b). Many ecotoxicology researchers have also used this weight-of-evidence approach to conduct sound ecological risk assessments (Burton [Ed.], 1992).

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Chapter 4
Quality Assurance/ Quality Control and Data Analysis

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THE NEED FOR TESTING QUALITY CONTROL AND STANDARDIZED DATA ANALYSIS IN BIOMONITORING

Toxicity results represent enforceable National Pollutant Discharge Elimination System (NPDES) permit criteria under the Clean Water Act in the same manner as do more traditional chemical determinations. In fact, effluent toxicity data are of equal or greater importance (from a regulatory viewpoint) than many conventional, nonconventional, and priority pollutant parameters listed in NPDES permits around the country. Related to enforcement issues is the real need to generate and report data that are defensible in a court of law. Proper quality assurance and control is as important in a toxicology laboratory as it is in an analytical chemistry laboratory. In either case, the objective is to attain a high level of reliability and completeness and, as a result, reduce

uncertainty. Completeness and reliability of historical data are two of the most important criteria to a successful defense of past practices.

The advent of the NPDES requirement for whole effluent toxicity testing in the early 1980s spawned a new service within the environmental consulting, engineering, and analytical industry—bioassay testing. The growth of this service since that time is analogous to the past explosion in environmental chemistry contract laboratories. While chemical testing has matured to a point of national and state certification programs and standardized quality assurance/quality control (QA/QC) methodologies, bioassay laboratories have no formal nationwide programs and relatively few state certification programs. As a result, the level of quality assurance and control varies widely. Until such time as the conditions change, individuals or organizations contracting for this service must exercise caution in evaluating and selecting aquatic toxicity testing laboratories.

This chapter provides several important considerations for selecting a laboratory to perform wastewater bioassay testing and biomonitoring data analysis. While good laboratory practice procedures for effluent toxicity testing are not specified by regulation, an appropriate level of quality control is necessary to ensure the reliability of test results.

False-positive results can trigger the need for toxicity identification and reduction evaluations; enforcement action; suits from public interest groups; and, ultimately, a conclusion that the receiving water is being significantly impaired. Conversely, false reports of no apparent toxicity or acceptable toxic levels can also be consequential insofar as delayed detection of a wastewater problem often results in more costly remedial actions.

BIOMONITORING TESTING QUALITY ASSURANCE/QUALITY CONTROL

Quality assurance is defined as the planned and systematic actions necessary to provide adequate confidence that an item or service will satisfy given needs. Quality control differs from quality assurance in that it is the process through which we measure actual quality performance, compare it with a standard, and act on the difference. To ensure that biomonitoring tests yield data that are both precise and accurate, careful QA/QC procedures should be followed. Documentation of all aspects of the processes that lead to results should be maintained. These items include sample chain-of-custody records, instrument logs showing maintenance and calibration records, quality control records of standards run on the instruments, and records of dilution water chemistry.

Any aquatic bioassay testing program should always have a complete set of documented QA/QC procedures. Such QA/QC maintenance applies to the

chemical analyses, the chemical data on common water constituents or water/sediment quality parameters, and test species' toxicological responses to known toxicants. Every testing protocol in use includes guidance in this area. The theory is based on the assumption that given a similar stock or source of a test species, under similar maintenance and testing procedures, and with the same quality of a stressor or toxicant, the test species should show appropriate inhibitory concentration/effective concentration/lethal concentration (IC/EC/LC) responses that are statistically consistent over time (ASTM, 1993). Reference toxicants have been established for estuarine and marine toxicity tests (U.S. EPA, 1989b and 1991, and U.S. EPA and U.S. ACOE, 1991).

Negative controls (or solvent controls) are also required for many estuarine and marine bioassays but are most relevant when used in sediment tests. Some liquid-media bioassays, however, may also require such extra controls in the form of regular laboratory-grade water, local ambient receiving water, or actual solvent-water mixtures. In sediment testing, a negative control typically consists of uncontaminated sediment that is acceptable to the test species. Likewise, if a solvent is used to dissolve some toxicant or mixture into a chosen test medium (water and/or sediment), then this solvent-control is made up of the same solvent concentration as is used in all treatments tested. In any case, the resulting acceptable survival or insignificant effects demonstrated by the test species in the negative control indicate whether sediment handling or testing procedures were also within acceptable parameters.

Another typical requirement in QA/QC for sediment tests is the use of reference sediments. This is analogous to the ambient receiving waters used in liquid-media tests. The reference sediment may be the same material as that of the negative control, should be uncontaminated, and is typically sediment that also comes from an area near that of the sampled (unknown) sediments. The reference sediment should have the same qualities as those of the sample sediment (such as grain size, water content, pH, interstitial-water salinity, and total organic carbon content). This is important for two reasons:

- The qualities of the sampled sediments, especially grain size, salinity, and water content, should be as similar to those of the reference sediment as possible because the test species used may not respond in the same manner to a much different sediment, and this could bias results; and
- The similarity of the reference sediment and sampled sediment means that the resulting data should be more defensible, they can represent a more site-specific analysis, and sampled sediment results can be compared to the reference sediment's background effects.

REFERENCE TOXICANT TESTS. Reference toxicant tests are run to determine the sensitivity of organisms being tested and evaluate general laboratory procedures. Test organisms are exposed to various concentrations of

U.S. Environmental Protection Agency- (U.S. EPA-) approved reference toxicants. These reference toxicants include sodium chloride, potassium chloride, cadmium chloride, copper sulfate, sodium dodecyl sulfate, and potassium dichromate. The degree of each organism's response to various concentrations of the reference toxicant is measured and compared to acceptable ranges. If organisms respond outside of acceptable ranges, test organisms are considered to be either too sensitive or not sensitive enough to be used in biomonitoring tests.

LABORATORY CAPABILITY REVIEWS

Evaluating the quality and capabilities of a new or previously unused laboratory can be simplified if a state certification or accreditation program exists. In some U.S. states, such as New Jersey or North Carolina, bioassay laboratories are required to demonstrate their competence by successfully conducting qualification testing with reference toxicants supplied by the state. These qualification tests are specific to the organism and type of bioassay test. A laboratory, for example, may be qualified by the state to conduct 96-hour acute fathead minnow tests, yet not be qualified to conduct 7-day chronic fathead minnow tests or any other type of bioassay test. In other U.S. states, such as Florida, laboratories are required to submit their standard operating procedures (SOPs) or quality assurance program plans for review by state officials before approval to conduct bioassay tests. These state review programs offer a means to screen candidate laboratories. In addition, many laboratories participate in the discharge monitoring report quality assurance (DMR QA) program sponsored by U.S. EPA. Unknown toxicants are sent to biomonitoring laboratories for analysis using the species and type of tests that the laboratory typically performs. Test results are compared to the known toxicity of the toxicant. Acceptable ranges are decided on and participants are notified of their performance. Biomonitoring laboratories that do contract work are required to submit to their clients the results of their performance on DMR QA samples. State regulators may require satisfactory performance on DMR QA samples as part of their certification requirements.

In addition to discussions with the appropriate state NPDES program office, permit holders should consider key attributes or characteristics of potential laboratories before awarding work. A list of considerations is provided in Table 4.1. The laboratory's location or proximity to your facility may be an important consideration. This is especially true when test requirements dictate limited holding times of fewer than 24 hours or when the wastewater to be tested has chemical, physical, or biological characteristics that may alter toxicological properties within a relatively short time period. However, for most permit requirements, laboratories routinely test samples shipped by

Table 4.1 Laboratory reviews: capabilities and points to consider

Certification and accreditation
Experience with audits
Physical laboratory
1. Size and location
2. Construction and cleanliness
3. Bench space
4. Water and aeration supply
5. Isolation capabilities
6. Temperature-controlled laboratory/environmental chambers
7. Sample holding facilities

Test apparatus
1. Diluters/glassware, etc.
2. Monitoring meters
3. Organism holding capabilities

Staff
1. Number and expertise of staff
2. Degrees and experience of technicians, study leaders, and management
3. Experience in conducting required tests
4. Staff training programs/documentation

Standard operating procedures
Test protocols
Data turn-around time

Quality control

Access and/or availability to study plans and SOPs
Quality assurance/quality control procedures used for data review
Availability of QC records
Sample tracking records (chain-of-custody forms)
Reference toxicant testing
1. Frequency
2. Records

Data collection and analysis procedures
Dilution water source and documentation of quality
Instrument calibration procedure
Report review and sign-off

overnight carrier, even though shipping costs for overnight service of large quantities of water can be significant.

Size and internal isolation capacity of the prospective laboratory should also be considered. If a laboratory operates at or above capacity with a limited ability to isolate tests, there exists the risk of cross contamination, or worse, inadvertent mixing of test samples. Moreover, there should be ample space in separate enclosed areas for sample storage, glassware washing, and test organism culturing. This is especially important for laboratories involved with

testing unknown pure compounds or other potentially toxic and/or poorly characterized materials.

The overall cleanliness of the building, laboratory facility, and office space is also important as is the need for uninterrupted utility service—specifically, heating and lighting. Given that laboratory tests are designed to simulate natural conditions as much as possible, lighting and temperature control are critical. To reduce laboratory-induced stress, some laboratories have adopted use of timed fluorescent bank lighting, whereby 24-hour light intensity cycles can be simulated. This regime includes regulating lighting to mimic dawn and dusk cycles.

Laboratory water supply is another extremely important factor that warrants careful attention. While unconditioned tap water should never be used directly as test diluent or culture water, it may be used, after charcoal filtration and deionization, to formulate reconstituted water, which is often used as culture water or diluent. Documentation of tap water quality may, therefore, be appropriate.

Aeration of test and control chambers is typically not acceptable because of the possibility of driving off volatile organics and/or the possibility of oxygenating, thereby effectively treating, test waters. Aeration of chambers is, however, acceptable and, in fact, required when dissolved oxygen falls below a stress threshold. Under such conditions, the source of air is important. Many of the larger laboratories use one or two central compressors for aeration to all laboratory and culture room chambers. The intake of these compressors must be free from airborne contaminants. Intakes, for example, located near loading docks or other motor vehicle usage areas may be a prime source of external contamination of the laboratory and may require filtration mechanisms. Compressors should also be equipped with seals designed to prevent oil contamination of the air lines.

Test apparatus and organism holding capabilities are also important. For obvious reasons, all materials used in bioassay testing or the culture of test organisms should be made of nontoxic and nonleachable components. Acceptable materials include lead-free glass, no. 316 stainless steel, unplasticized polyethylene or polypropylene, polytetrafluoroethylene, vinyl chloride and vinyl acetate copolymer, and fiber glass. Connectors and tubing known to absorb trace organic compounds should be used once and discarded.

The quality of a laboratory's staff is perhaps the most critical consideration. The quality and experience of the laboratory staff have become even more important over the last few years coincident with requirements for use of chronic testing with sensitive endpoints, including organism growth, reproduction, and behavioral effects. Another significant factor involves staff structure and responsibilities. Interviews with bench technicians regarding their responsibilities and understanding of organism handling and test requirements are equally as important as discussions with laboratory management. Bench

technicians and scientists are the "hands-on" people who perform the tests. Their competence determines the reliability of test results.

Related to the quality of the laboratory staff is the presence or absence of clear laboratory directives on how tests are to be performed, proper acclimation and handling of test organisms, meter calibration procedures, data handling and processing, and all other laboratory activities. As in analytical chemistry laboratories, these directives should be included in printed SOPs. Not only is their existence important, but all laboratory personnel should have training in their implementation and accessibility to their directives.

Given the importance of obtaining reliable results or, alternatively, the costs of repeating poorly designed tests, it may be advisable to contract a specialist to conduct laboratory screening audits, work with the contract laboratory in designing appropriate tests, and review the quality of test results carefully. One source for information regarding laboratory evaluation procedures is *Manual for the Evaluation of Laboratories Performing Aquatic Toxicity Testing* (Klemm *et al.*, 1990).

LABORATORY QUALITY ASSURANCE/QUALITY CONTROL

It is virtually impossible for a laboratory to offer a high level of reliability in test results without maintaining a sound QA/QC program. Historical records should include at least the following:

- Sample tracking records, including all chain-of-custody forms;
- Sample holding documentation;
- Records on sample manipulations (for example, operation);
- Instrument maintenance and calibration records;
- Test organism history records, including their source, life history, quarantine, and acclimation records;
- Reference toxicant data, including records of all tests, control charts, and frequency of testing;
- Records of all audits and documentation of corrective actions;
- Records of the source of laboratory-grade diluent and control waters;
- Records of all in-house biological cultures, including brood stock sources and diversity and culture maintenance; and
- Appropriate senior review and QA sign-off of all test reports.

While not totally inclusive, the above list represents key QA/QC records that laboratories should maintain to provide defensible results.

Bioassay laboratories are not dissimilar to analytical chemistry laboratories with regard to the importance of controls and replicates. Proper use of multiple controls is an effective means to ensure that test organisms' responses

within treatment chambers are reliable. Reliability is further enhanced with use of an adequate number of replicates. Increasing the number of replicate chambers, while increasing the test population, strengthens the statistical basis for estimating the actual response and improves the predictive power of the test itself. Testing guidelines provide recommended minimum numbers of replicate chambers and test populations. One is not restricted to these minimums, and the larger the test population or sample number, the more reliable the results. These statistical rules of thumb apply to all sciences; however, they are especially relevant to biological sciences due to the inherent variabilities between individuals and their subsequent differences in responding to environmental stimuli.

Finally, it is important to remember that the goal of toxicity testing is to reduce uncertainty to a confidence level that is acceptable to the appropriate regulatory authority and the discharger in a cost-effective manner. To this end, test programs and the level of QA/QC efforts should be determined jointly between the client and the contract laboratory.

Data Analysis

The defensibility of test results for permit compliance or establishment of water quality criteria depends on reliable data analysis. Standard methods for statistical analysis and data presentation are discussed below.

ACUTE DATA ANALYSIS. Acute tests use mortality as an endpoint. For an acute test to be considered valid, the minimum rate of control organism survival must be 90% or greater. When adequate control survival is achieved and the reference toxicant tests for the organisms fall within the acceptable range, test data can be accepted as reliable. If dilutions are used and they span the correct range of observed mortality, an LC_{50} can be determined. The LC_{50} represents the statistically derived percent dilution that will yield 50% mortality (will kill half the organisms). The LC_{50} can be calculated using a graphical method that involves semilogarithmic plotting of percent mortality versus percent effluent or by using a computer software program designed for this purpose. Another value, the no observed effect concentration (NOEC), represents the highest effluent concentration at which survival is not significantly different from that of the control. The NOEC is determined by hypothesis testing, which will be explained in the next section on chronic toxicity data analysis. The Fisher's Exact Test can be used to compare groups of organisms exposed to effluent to a control group to determine if there is a statistically significant difference in survival rate.

CHRONIC DATA ANALYSIS. Data gathered during chronic toxicity tests encompass sublethal parameters. Rather than concentrating only on mortality, chronic tests focus on hatchability, gross morphological abnormalities,

growth, weight gain, reproductive success, and cell growth stimulation. There are several ways to analyze chronic data to determine a test endpoint. One commonly used technique involves hypothesis testing. Hypothesis testing compares data for each effluent dilution to the control and asks the question, "Is the effect in this dilution statistically different from that in the control?" This method is used to determine the lowest dilution that shows no statistically significant difference from the control. Statistical significance can be determined for either 95 or 99% confidence levels. This dilution represents the NOEC or the no observable effect level (NOEL). The lowest observable effect concentration (LOEC) represents the lowest dilution in which an effect is observed. Other common terms derived from hypothesis testing include the chronic value (ChV), defined as the geometric mean of the NOEC and the LOEC, and the maximum allowable toxicant concentration (MATC), which is equivalent to the ChV. Several statistical tests used for hypothesis testing are shown in Table 4.2. The statistical method used must be suitable for data being analyzed. The statistical procedure cannot be selected before the test is conducted because data generated must meet certain criteria for each test. Parametric methods analysis of variance (ANOVA), Dunnett's, and probit require that test data meet normality assumptions and that the variances of the different treatment groups within a test be homogeneous.

Table 4.2 Statistical tests used for hypothesis testing

Test name	Type of test	Data requirements[a]
Dunnett's test	Parametric	A,B,C
Bonferroni's *t*-test	Parametric	A,B
Steel's test	Nonparametric	C
Wilcoxon rank sum test	Nonparametric	

[a] A = normal distribution, B = equal variances between treatments, and C = equal number of replicates between treatments.

Chronic data typically are analyzed with ANOVA statistical tests, such as Dunnett's test, which establish with statistical confidence limits and levels the treatment concentration at which test species' responses were significantly different from that seen in the control species/treatment. It is recommended, however, that a good biostatistical reference be consulted as well (for example, Zar, 1984). Many computerized statistical and data management systems specific for aquatic bioassays are available for a modest investment (SETAC, 1994). Standard aquatic testing protocols also have standard statistical methods and flow charts to aid in the validation of data derived from such bioassay tests (ASTM, 1993; U.S. EPA, 1989b and 1991; and U.S. EPA and U.S. ACOE, 1991).

A second method for the analysis of chronic data uses a different statistical technique for determining *point estimates* of effluent effects. Rather than checking for levels at which effects disappear, this technique seeks to estimate a percentage reduction in survival or growth caused by the effluent compared to the control. Point estimate terms include the LC, EC, and IC. The LC was discussed in the acute test section as the LC_{50}. Similarly, an EC_{50} would be the concentration of an effluent at which 50% of the organisms exhibit a particular effect. Any numerical variation of the percent of the population involved can be expressed in the point estimate method. The ICx represents the inhibition concentration with an effect of x%. An example would be that an IC_{25} for reproduction is an estimate of how much chemical or wastewater caused a 25% reduction in reproduction in test organisms. A strong advantage of the point estimate technique as opposed to hypothesis testing is that statistical confidence intervals can be calculated for the toxicity endpoint. This method also results in measurements that are not as dependent on the test dilution used. In many situations, the IC_{25} is approximately equal to the NOEC determined by hypothesis testing. Commercially available software can be helpful for calculating chronic toxicity test endpoints. Many of these software applications guide the user in the most appropriate statistical test to use.

Toxicity endpoints can be translated into toxic units (TU). A TU is defined as the inverse of a given endpoint. For example, an NOEC of 5% effluent results in a TU of 20 (1/0.05). This unit of measurement may be more easily understood because it directly corresponds to toxicity (a higher TU means a higher toxicity level).

As mentioned earlier, in some tests, it is not unusual to observe chronic toxicity tests in which the organisms in test waters have better growth or reproduction than in the controls. This can occur in cases where the test water contains microorganisms that provide a better food source than the almost sterile control water. In algal growth tests, effluents that contain higher levels of beneficial micronutrient (especially phosphorus) will have growth that exceeds levels of controls.

The most common chronic data endpoints used are the ANOVA-determined significant effects of the NOEC and the LOEC. The LOEC is the lowest treatment concentration in a test that is significantly different (in test species' responses) from that seen in the control (control species' responses). The NOEC is the highest treatment concentration in a test that is not significantly different in test species' responses from that seen in the control.

Visualize, for example, an array of six test concentration treatments arranged in an increasing geometric series from a control treatment (0%) to the highest concentration treatment tested (for example, control, 1, 3, 10, 30, and 100% sample concentrations). The ANOVA and other statistical analyses will indicate particular test treatments that were significantly different in response from the control treatment. Suppose that all sample treatments above except the 1% sample treatment were found by ANOVA to be significantly

different from the control treatment. In this case, the second sample treatment (3%) is the *lowest affected* sample treatment and would be the LOEC. Consequently, the first sample treatment (1%) is the *highest unaffected* or *nonsignificant* sample treatment and would be the NOEC. It is possible for all sample treatments to be labeled as significantly different. In this case, the first and lowest sample treatment (1%) would be the LOEC and the control treatment would be the NOEC. Theoretically, the *negative* or *significant* responses exhibited by the test species would start to occur at some concentration point between the treatment concentration of the NOEC and that of the LOEC. This estimated concentration is called, by convention, the MATC. The point estimation of the MATC is called the GMATC (formerly, "critical value/ChV") and is the geometric mean of the NOEC and LOEC values. In the first sample above, the LOEC is the 3% sample treatment and the NOEC is the 1% sample treatment. Thus, the MATC lies between 1 and 3% sample treatment, and the GMATC would be the geometric mean of 1 and 3% (U.S. EPA, 1989b and 1991). The GMATC is used, to some extent, in the field of ecotoxicology for ecological risk assessment (ERA) work. However, most environmental regulations, water and sediment quality criteria derivations, and ERA estimates of conservative risk are based on NOEC data whenever possible. The NOEC data, by nature, yield the most conservative estimates of ecological toxicity or adverse effects, and use of the NOEC for ERA criteria means that, theoretically, more types of species and their younger age classes may be at less risk to adverse anthropogenic effects (Marshack, 1991, and U.S. EPA, 1992).

In general, true acute data in the form of mortality data (LC) versus toxicant concentration data can be analyzed with an array of statistical tests such as moving-average-angle, binomial, or probit methods. Such LC data can result from screening acute tests, multitreatment acute tests, or more sensitive chronic tests. Guidance documents may allow the use of LC or even EC/IC data when LOEC or NOEC data are lacking so as to provide the best estimate of toxicity data available (U.S. EPA, 1989a). Other testing standards also refer to some EC, IC, or LC low-percent-affected (1 to 49% affected) concentration data similar to those of an NOEL from the same type of test and species. These criteria are based on what are considered "acceptable effects" and do vary with species and regulations. Such data are also based on powerful statistical analyses (probit, regression, power analysis) that are run on computers. Such computer statistical programs can give one a percent-organism-affected for any given toxicant or stressor concentration and can allow extrapolation between and beyond the actual tested concentrations to yield EC, IC, and LC values. A general hierarchy of which endpoint data are best to use for estimating ecological risk based on the collective use of available NOEC, LOEC, IC, EC, and LC toxicity data can be arranged from most to least conservative as follows: (1) NOEC data, (2) GMATC data, (3) LOEC data, (4) low (1 to 49%) IC/EC data, (5) IC_{50}/EC_{50} data, (6) low (1 to 49%) LC data, and (7) LC_{50} data. The debate concerning what are acceptable adverse effects

or mortalities is continuing and is beyond the scope of this chapter or handbook. However, such conservative IC/EC/LC to NOEL estimations should not be done unless clear protocol allows for them and they are based on sound statistical and ecological reasoning.

The NOEL data are the best estimates of safe effects. However, ecotoxicological data do not always provide direct NOEL values, depending on the types of tests, species tested, and differential mortality or sublethal effects that are measured. Typically, there are more mortality-related data than no-effect data because of the latter's more expensive and extensive bioassay procedures. In an attempt to account for data that are not of a no-effect nature, it has been a standard practice in the field of human and ecological toxicology to adjust toxicological endpoint data by a safety factor (SF). An SF, in effect, adjusts the original IC, EC, or LC data value to a level that could be professionally judged as similar to an NOEC or no-effect level, *if* an actual bioassay to determine the NOEC had been done. This practice was first used by U.S. EPA and other regulatory agencies for deriving human health no-effect levels from animal and plant toxicity bioassay data. This was done because most data on anthropogenic or adverse effects could only be derived from animal and plant bioassay systems, as such testing both was cost-effective and did not involve testing humans. The use of the SF incorporates ecologically sound protocols for determining an SF's magnitude when toxicity data are compared between different taxonomically related groups. Marine and estuarine bioassays are good tools for estimating, establishing, or confirming ecotoxicity SFs. A good description of how to derive ecologically sound safety factors can be found in *Performing Ecological Risk Assessments* (Calabrese and Baldwin, 1993).

ARTIFACTUAL TOXICITY

Artifactual toxicity can be defined as any measurable adverse effect that results during a biomonitoring test and is caused by factors other than water quality. Some of the causes of artifactual toxicity include improper sample collection and storage; stress caused by rough handling of test organisms; aeration turbulence in the test container; and differences in micronutrient in culture water, dilution water, or test water. Missing essential ions can cause physiological problems that are not considered toxic effects. This type of artifact has been seen in selenium-poor waters in *Ceriodaphnia* chronic tests. Test results in which artifactual toxicity is suspected should not be used for NPDES permit compliance.

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Chapter 5
Watershed/Regional Assessment and In-Stream Monitoring

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Introduction

The purpose of the Clean Water Act is to protect both human health and the condition of biological organisms that live in surface waters. Study is needed to protect biological organisms, including laboratory and field toxicity testing, characterization surveys, ecological risk and impact assessments, and water quality and ecosystem modeling exercises. However, most people probably

think of laboratory toxicity testing when they first hear the word "biomonitoring." This is because most of the regulatory activity for water quality relies on laboratory toxicity testing. *Biological monitoring* is now used to refer to the gathering of biological information in both the laboratory and the field for the purposes of making some sort of environmental decision or determining whether regulatory standards and criteria are being met (Cairns, 1990). This knowledge base must be extended to a broader context to protect aquatic ecosystems, and this chapter presents some of the issues that should be considered: biological criteria, in-stream biological monitoring, and watershed/regional assessment needs with regard to data and methods.

The Clean Water Act has an objective of *biological integrity.* Karr and Dudley (1991) defined biological integrity as ". . . the ability of an aquatic ecosystem, to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitats of a region." Allan and Flecker (1993) propose six factors as being of critical importance to biodiversity in running water: habitat loss and degradation, the spread of exotic species, overexploitation, secondary extinctions, chemical and organic pollution, and climate change. Judy *et al.* (1984), Miller *et al.* (1989), and Williams *et al.* (1993) reported that stresses other than chemical or point source pollution were most significant with regard to water quality degradation for aquatic animals. Miller *et al.* (1989) attributed North American fish extinctions to physical habitat alteration (73%), introduced species (68%), and chemical habitat alteration (38%). Thus, it is apparent that laboratory toxicity testing that addresses stresses from pollutants is only one of the pieces of information needed to evaluate ecological integrity. While pristine or unimpaired water bodies no longer exist, least affected conditions can be used to guide restoration and protection programs (Davis and Simon [Eds.], 1995). Biological criteria are "numeric values or narrative expressions that describe the reference (least impacted) biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use" (U.S. EPA, 1990). Over the years, environmental scientists have tried to address questions such as the following:

- Can tests on a few organisms represent all organisms?
- Can laboratory tests be representative of effects experienced in the field?
- Can microcosm/mesocosm tests better represent effects on ecosystems?
- Can we remotely sense biological responses to toxicants in the field?
- What are the advantages and disadvantages of single versus multimetric indicators?

Much of environmental science has been devoted to monitoring the environment for purposes of surveillance rather than to support specific

assessments, and this has led to significant criticism (NRC, 1990; Perry *et al.*, 1987; and Segar and Stamman, 1986). Suter *et al.* (1993) identify at least four general purposes for environmental surveillance:

- Assessing statutory or regulatory compliance;
- Providing information needed for systems control or management;
- Assessing environmental quality to determine whether it is satisfactory; and
- Detecting unexpected environmental damage.

Ecologists and many natural resource managers recognize that often the environmental hazards or stresses that we currently have to address (for example, acid deposition, nonpoint source pollution, introduction of exotic species, and increased global carbon dioxide alteration of hydrologic systems) affect large geographic areas. Yet, traditional concepts and methods in ecology and assessment are relevant mainly to single sites or small geographic areas. Also, monitoring programs and assessment needs often have not been integrated, and rigorous quantitative assessments have been the exception rather than the norm for large geographic areas. This chapter gives an introduction to fundamental issues for watershed and regional assessment and identifies the needs for physical, chemical, and biological monitoring and research to be designed and integrated to support such assessments.

REGIONAL OR WATERSHED APPROACH TO MANAGING WATER QUALITY AND AQUATIC ECOSYSTEMS

This section focuses on assessment approaches and issues of scale for management of water quality and aquatic ecosystems. Examples from current research and government programs are used. Detailed discussion of assessment approaches is restricted to risk assessment. Several other assessment approaches are relevant to biological monitoring and are briefly mentioned. Naiman *et al.* (Eds.) (1995) set out to establish a research framework, *The Freshwater Imperative*, with two key attributes: "the primacy of regional scale efforts and the clear recognition that the development of predictive capabilities is essential if we are ever to cope successfully with unforeseen events, or to develop what today are called 'adaptive' management regimes."

ORGANIZING PARADIGMS. When one moves from monitoring and analyzing data at the site-specific or local scale to the watershed or regional scale, it is not obviously apparent what is the appropriate resolution for data or how

to bound the activity with regard to spatial and temporal scales. Regional management requires organizing paradigms or conceptual models, and an assessment framework can serve this purpose; risk assessment is used as an example here. Spatial scale can also serve as a strong organizing paradigm for regional management and is compatible with a risk assessment framework (Hunsaker and Graham, 1991).

Risk Assessment. Society needs a quantitative and systematic way to estimate and compare the effects of human activities on the environment. Beyond regulatory discharge requirements, assessments provide the purpose or context for biomonitoring. Different approaches for environmental assessments have developed over time (Hildebrand and Cannon, 1993, and Westman, 1985) with much of the impetus coming from the requirements of the National Environmental Policy Act of 1969 (NEPA). Biomonitoring should be designed to support risk assessment as a fundamental component of environmental decision making because the cost of eliminating all environmental effects of human activities is impossibly high, and regulatory decisions must be made on the basis of incomplete scientific information (Moghissi, 1984, and Ruckelshaus, 1983). "Risk assessment can be defined as *the process of assigning magnitudes and probabilities* to the adverse effects of human activities or natural catastrophes. This process involves identifying hazards such as the release of a toxic chemical to surface waters that support fisheries, and using measurement, testing, and mathematical or statistical models to *quantify the relationship between the initiating event and the effects*" (Suter *et al.*, 1993). Suter *et al.* (1993) list several advantageous properties of risk assessment:

- It provides the quantitative bases for comparing and prioritizing risks. If, as is typically the case, all alternatives have some hazardous properties, it is not possible to make a choice without characterizing the risks.
- It provides a systematic means of improving the understanding of risks. Research can be prioritized by identifying and comparing the uncertainties associated with the characterization of the different steps in the causal chain, from the initiating event to the effect.
- By expressing results as probabilities, risk assessment acknowledges the inherent uncertainty in predicting future environmental states, thereby making the assessment more credible.
- Risk assessment estimates clear consistent endpoints such as cancer death rates or probability of bankruptcy. This is in contrast to other approaches to ecological assessment that have unstated or ambiguous endpoints such as ecosystem integrity.
- By basing the estimates of probability and magnitudes of effects on formal quantitative methods, risk assessment provides a means for parties making environmental decisions to compare the implications of their assumptions and data rather than negotiating on the basis of polit-

ical influence. The openness and consistency of such methods ensures fairness and allows thorough scientific review of the bases for decisions. Differences in results expressed as magnitudes and probabilities of effects can be compared to determine whether they have different implications, given costs and benefits of alternative actions.

- Risk assessment separates the scientific process of estimating the magnitude and probability of effects (risk analysis) from the process of choosing among alternatives and determining acceptability of risks (risk management). The result of this separation is reduced likelihood of analyses that are biased to fit desired decisions and greater credibility for both the technical and the policy sectors.

The U.S. Environmental Protection Agency (U.S. EPA) has published an ecological risk assessment framework (U.S. EPA, 1992a) and several supporting documents (U.S. EPA, 1993a), which are the work of many agency and outside scientists over several years (Figure 5.1). While the historical focus has been on risks from chemicals, the framework accommodates evaluation of risks from biological and physical events also. In addition, it recognizes the need for both retrospective and predictive risk assessments. Both site-specific and predictive risk assessments have dominated regulatory agencies and the projects used in the development of ecological risk assessment methods. However, emphasis has been shifting to assessments of the effects of actions that began in the past and may have ongoing consequences such as waste sites, acid rain, introduction of exotic species, and long-lived chemicals. These assessments, which are termed *retrospective*, are distinguished by two facts: the scope of the problem has been defined by events, and there is a polluted/affected environment to be examined and measured. These facts make retrospective assessments more diverse than predictive assessments in both their methods and logic (see Suter *et al.*, 1993, for more details).

Because many environmental hazards (for example, acid deposition or nonpoint source pollution) affect large geographic areas, the factors that are similar and different between local (site-specific) and regional risk assessment have been identified (Hunsaker *et al.*, 1990) and illustrated (Graham *et al.*, 1991). Regional risk assessment has some attributes in common with local risk assessment but has others that are unique. The general theoretical framework for doing the two types of environmental risk assessment is the same. Both have two phases: first, the *hazard definition*, in which the *endpoint*, *source terms*, and *reference environments* are defined and described; and second, the *problem solution*, in which the exposure and effect on the endpoint are assessed by using models, and the risk and its associated uncertainty are determined (Table 5.1). Regional risk assessment differs from local risk assessment in two ways: the extent of interaction between source terms, endpoints, and reference environment and the degree to which boundary definition and spatial heterogeneity are significant in determining uncertainty.

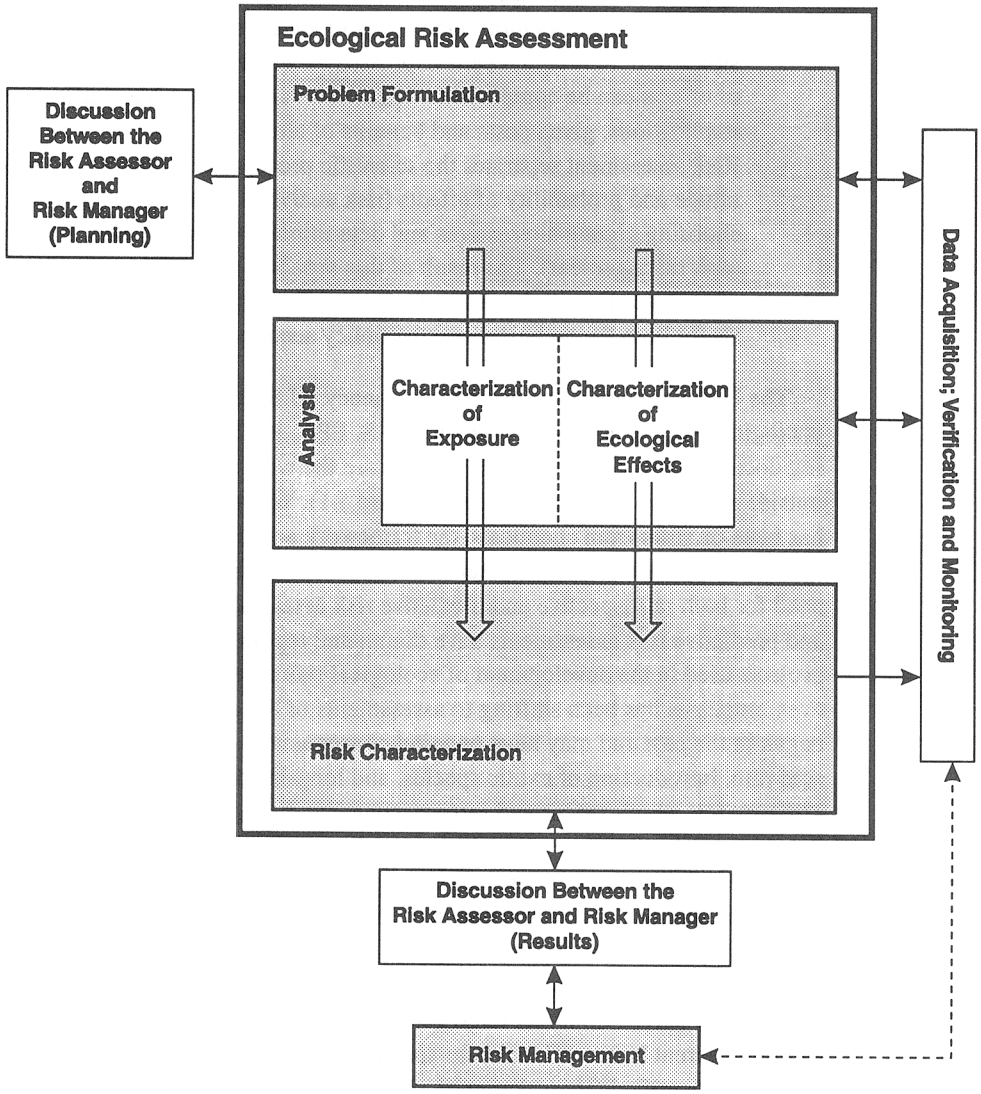


Figure 5.1 Framework for ecological risk assessment

Although local risk assessments involve the development of databases and the use of models, these steps may be more significant in regional risk assessments. Few regional-level databases of biological variables exist; furthermore, unique problems arise in aggregating or integrating dissimilar local data into regional databases. Regional models of ecological processes are less common and can be difficult to validate. Hunsaker and Graham (1991) illustrate the influence of boundary definition and spatial heterogeneity on modeling acidic deposition effects on Adirondack lakes. U.S. EPA's Environmental Monitoring and Assessment Program (EMAP) is a program purported to support regional ecological risk assessment (see discussion of federal programs that follows).

Table 5.1 **Regional risk assessment terms using the Piedmont physiographic region as an example (reprinted from Hunsaker, C.T., *et al.* [1990] Assessing Ecological Risk on a Regional Scale. *Environ. Manage.*, Vol. 14, 325, with permission from Springer-Verlag, New York, N.Y.)**

Term	Definition	Example
Hazard	Pollutant or activity and its disruptive influence on the ecosystem containing the endpoint	Forest cutting practices that eliminate critical habitat for an endangered species
Endpoint	Environmental entity of concern and the descriptor or quality of the entity	Extinction of an endangered species
Source terms	Qualitative and quantitative descriptions of the source of the hazard	Forest cutting practices and the laws and economic factors that influence them in the Piedmont
Reference environment	Geographic location and temporal period for the risk assessment	Piedmont of the U.S. in the next 10 years
Exposure/habitat modification	Intensity of chemical and physical exposures of an endpoint to a hazard	Amount of habitat, for an endangered species, that is lost

Other Assessment Approaches. U.S. federal or state officials, acting as trustees for natural resources, can seek compensation under the Clean Water Act or Comprehensive Environmental Response, Compensation and Liability Act for damage to natural resources caused by releases of oil or other toxic materials. *Damage assessments* have typically been *ad hoc* performances, but a modeling system has been developed by the U.S. Department of the Interior for natural resource damage assessments (U.S. DOI, 1987a and 1987b). Assessments that determine the suitability of ecosystems as habitat for a species are termed *habitat assessments*. The U.S. Fish and Wildlife Service's habitat evaluation procedure provides a framework for determining habitat quality for specific fish and wildlife species (U.S. FWS, 1980) and has been extended to consider pollution effects (U.S. DOI, 1987b). Use attainability analyses performed under the Clean Water Act also function as habitat assessments (U.S. EPA, 1983). The Environmental Requirements and Pollution Tolerance System is a large relational database on habitat requirements of aquatic species in the upper midwestern U.S. that allows inference about water quality from the species present (Dawson and Hellenthall, 1986).

Environmental impact assessment (EIA) is one of the oldest and most diverse areas of assessment because of the breadth of the activities that are considered "major federal actions" under NEPA and the use of multidisciplinary teams (for more information, see Beanlands and Duinker, 1983; Hildebrand and Cannon, 1993; and Westman, 1985). While EIAs have largely relied on deterministic methods, the requirement that uncertainties be presented suggests that quantification of uncertainty through the use of a risk assessment approach would be progressive.

Hazard assessment is the most commonly used methodology for analyzing the effects of chemicals on the natural environment. In essence, it consists of comparing the expected environmental concentration and the estimated toxic threshold and making a judgment as to whether the proposed release is safe, hazardous, or not sufficiently characterized for a decision. Hazard assessment was developed primarily by aquatic toxicologists and is based on an iterative process of testing and assessment called tiered testing (Cairns *et al.*, 1978). *Integrated assessment* is a process that can be used to evaluate and clarify resource management policy options and outcomes for decision makers (ORNL, 1995).

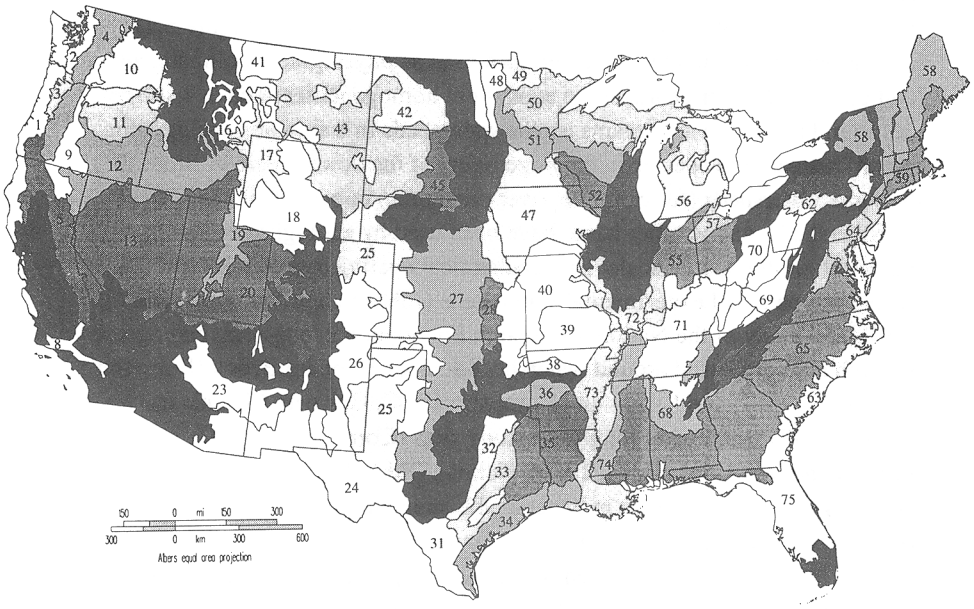
> The defining characteristics of integrated assessment are: (1) its focus on providing information and analysis that can be understood and used by decision makers rather than for merely advancing understanding, and (2) its multidisciplinary approach, using methods, styles of study, and considerations from a broader variety of technical areas than would typically characterize studies produced from a single disciplinary standpoint. Integrated assessment may combine scientific, social, economic, health, and environmental data and models.

Spatial Scale. As the issues being addressed by ecological research and resource management have become more complex and integrative, an interesting question has surfaced with regard to the spatial construct we use to characterize regions. Geographers have struggled for a long time with spatial interrelationships and questions of geographic characterization. A regional characterization scheme is especially difficult for aquatic ecosystems as two accepted approaches exist, *watersheds* and *ecoregions*, and both can be hierarchically constructed. An ecoregion is an area (region) of relative homogeneity based on one or more attributes in ecological systems. A watershed is an area of land draining to a specific point on a stream or to a lake or wetland; watersheds are based on topography and on the fact that water flows downgrade because of gravity. Watersheds are a logical analysis unit if chemical pollutants from point sources are the primary concern because the distribution and concentrations of these pollutants are primarily governed by the hydrologic network and dilution. However, if nonpoint source pollution (which is

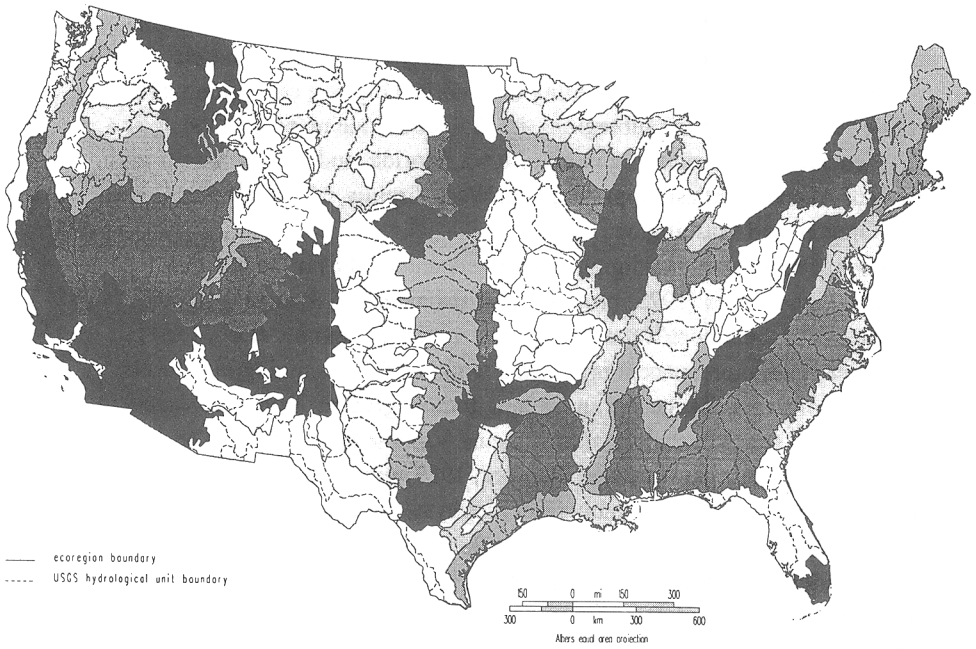
governed by land-use pattern, geology, and soils) and ecosystem condition as measured by aquatic organisms are of primary interest, then ecoregions (Gallant *et al.*, 1989) may serve as more useful analysis units (Figure 5.2). Ecoregions are defined by characteristics such as physiography, climate, and land cover and better characterize ecosystem homogeneities such as fish species found in headwater streams across watersheds in a similar physiographic region compared to species in the large rivers of the downstream portions of the same watersheds (for example, river continuum concept [Figure 5.3]).

Watersheds, on the other hand, have been used by ecologists as an organizing paradigm for spatial scale. Biogeochemical cycling research is typically carried out on small experimental watersheds (Johnson and Van Hook [Eds.], 1989), and many of the National Research Foundation's long-term ecological research sites are based around watersheds such as Hubbard Brook (Bormann and Likens, 1981, and Likens and Bormann, 1974), Coweeta (Swank and Crossley, 1987), and other long-term study sites (Correll [Ed.], 1986, and Johnson and Van Hook [Eds.], 1989). Naiman (Ed.) (1992) presents current knowledge and future trends in watershed management and research with a focus on watershed and regional models, indicators of environmental change, and new techniques for integrated resource management. Ecoregions have received more attention recently (Bailey, 1976, and Wiken, 1986). Omernik (1987) developed a set of ecoregions (1:7 500 000-scale map) for the conterminous U.S. that he believes are better suited to regional assessments for aquatic ecosystems than are watersheds such as the U.S. Geological Survey's hydrologic units (Omernik, 1995) (Figure 5.2b). Several U.S. states are using ecoregions for establishing variable water quality criteria (for example, Ohio, Arkansas, and Minnesota), including biocriteria. Ongoing ecoregion work to refine ecoregions, define subregions at 1:250 000-scale maps, and locate sets of reference sites is occurring in all of Iowa, Florida, and Massachusetts and parts of Alabama, Mississippi, Virginia, West Virginia, Maryland, Pennsylvania, Oregon, and Washington.

Several ecological paradigms about rivers emerged over the last two decades since Hynes (1975) proposed that the nature of streams is tightly coupled with catchment/watershed characteristics (Cummins *et al.*, 1984). The river continuum concept provided a template for examining how biotic attributes of rivers change within the longitudinal gradient from headwaters to outlets at the sea (Minshall *et al.*, 1985, and Vannote *et al.*, 1980). The serial discontinuity concept (Ward and Stanford, 1983) provided a construct for the likelihood of rivers to predictably reset biophysical attributes in relation to distance downstream from in-channel impoundments. Comparison of organic matter budgets in streams in different biomes provided the basis for the riparian control concept and demonstrated the importance of wood and leaves in lotic (running-water) systems (Gregory *et al.*, 1991; Harmon *et al.*, 1986; and Ward *et al.*, 1990). The nutrient spiraling concept (Newbold *et al.*, 1983, and Webster and Patten, 1979) led to an understanding of how plant nutrients are transformed from dissolved to particulate states during movement from


150 0 mi 150 300
300 0 km 300 600
Albers equal area projection

(a)


ecoregion boundary
USGS hydrological unit boundary
150 0 mi 150 300
300 0 km 300 600
Albers equal area projection

(b)

Figure 5.2 **(a) Omernik ecoregions (Omernik, 1987) and (b) ecoregions with U.S. Geological Survey hydrologic units (Omernik and Gallant, 1990); hydrologic units are a set of designated watersheds for the U.S. (1, Coast Range; 2, Puget Lowland; 3, Willamette Valley; 4, Cascades; 5, Sierra Nevada; 6, Southern and Central California Plains and Hills; 7, Central California Valley; 8, Southern California Mountains; 9, Eastern Cascades Slopes and Foothills; 10, Columbia Plateau; 11, Blue Mountains; 12, Snake River Basin/High Desert; 13, Northern Basin and Range; 14, Southern Basin and Range; 15, Northern Rockies; 16, Montana Valley and Foothill Prairies; 17, Middle Rockies; 18, Wyoming Basin; 19, Wasatch and Uinta Mountains; 20, Colorado Plateaus; 21, Southern Rockies; 22, Arizona/New Mexico Plateau; 23, Arizona/New Mexico Mountains; 24, Southern Deserts; 25, Western High Plains; 26, Southwestern Tablelands; 27, Central Great Plains; 28, Flint Hills; 29, Central Oklahoma/Texas Plains; 30, Edwards Plateau; 31, Southern Texas Plains; 32, Texas Blackland Prairies; 33, East Central Texas Plains; 34, Western Gulf Coastal Plain; 35, South Central Plains; 36, Ouachita Mountains; 37, Arkansas Valley; 38, Boston Mountains; 39, Ozark Highlands; 40, Central Irregular Plains; 41, Northern Montana Glaciated Plains; 42, Northwestern Glaciated Plains; 43, Northwestern Great Plains; 44, Nebraska Sand Hills; 45, Northeastern Great Plains; 46, Northern Glaciated Plains; 47, Western Corn Belt Plains; 48, Red River Valley; 49, Northern Minnesota Wetlands; 50, Northern Lakes and Forests; 51, North Central Hardwood Forests; 52, Driftless Area; 53, Southeastern Wisconsin Till Plains; 54, Central Corn Belt Plains; 55, Eastern Corn Belt Plains; 56, Southern Michigan/Northern Indiana Till Plains; 57, Huron/Erie Lake Plain; 58, Northeastern Highlands; 59, Northeastern Coastal Zone; 60, Northern Appalachian Plateau and Uplands; 61, Erie/Ontario Lake Plain; 62, North Central Appalachians; 63, Middle Atlantic Coastal Plain; 64, Northern Piedmont; 65, Southeastern Plains; 66, Blue Ridge Mountains; 67, Central Appalachian Ridges and Valleys; 68, Southwestern Appalachians; 69, Central Appalachians; 70, Western Allegheny Plateau; 71, Interior Plateau; 72, Interior River Lowland; 73, Mississippi Alluvial Plain; 74, Mississippi Valley Loess Plains; 75, Southern Coastal Plain; 76, Southern Florida Coastal Plain)**

upstream to downstream reaches. The ecotone concept (Holland *et al.* [Eds.], 1991, and Naiman and Decamps [Eds.], 1990) elevated interest in the importance of transformations and fluxes of materials that occur within boundaries between functionally interconnected patches that form the riverine landscape. Stanford and Ward (1992) believe that the ecotone concept integrates the other paradigms by emphasizing the functional connectivity inherent in all ecosystems.

Expanding on the river continuum concept illustrates the importance of using knowledge about spatial scale in the planning and execution of

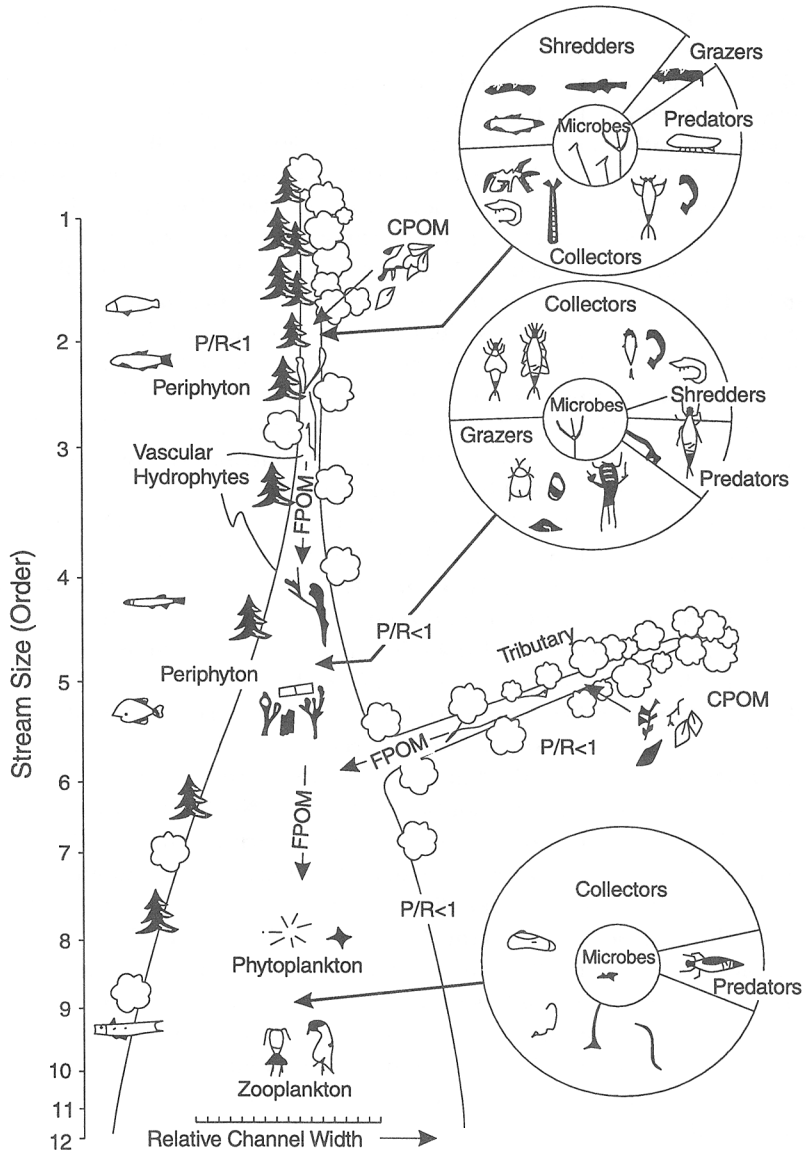


Figure 5.3 **River continuum concept as illustrated by relationship between stream size and the progressive shift in structural and functional attributes of lotic communities (P/R = ratio of gross primary productivity to community respiration; CPOM = coarse particulate organic matter; and FPOM = fine particulate organic matter) (Vannote *et al.*, 1980)**

assessments and monitoring. Vannote *et al.* (1980) proposed that the network of streams in a river drainage system is a continuum of physical gradients and associated biotic adjustments (Figure 5.3). They proposed headwater streams (orders 1 to 3), medium-sized streams (4 to 6), and large rivers (greater than 6). Many headwater streams are influenced strongly by riparian vegetation, which reduces autotrophic production by shading and contributes large amounts of allochthonous detritus. As stream size increases, autochthonous primary production and organic transport from upstream become more important compared to terrestrial organic input. This change is thought to be reflected in the ratio of gross primary productivity to community respiration being less than one in headwaters and equal to one in medium-sized streams. This conceptual model allows generalizations concerning the magnitude and variation through time and space of the organic matter supply, the structure of the invertebrate community, and resource partitioning along the length of the river. The river continuum concept views streams as longitudinally linked systems where system-level processes (cycling of organic matter and nutrients, ecosystem metabolism, net metabolism) in downstream areas are linked to in-stream processes in upstream areas. Thus, streams can be viewed as spatially heterogeneous systems.

Landscape ecologists seek to better understand the relationships between landscape structure and ecosystem processes at various spatial scales. The word *structure* refers to the spatial relationships of ecosystem characteristics such as vegetation, animal distributions, and soil types. *Processes* or *function* refers to interactions—that is, the flow of energy, materials, and organisms—between spatial elements. Because landscapes are spatially heterogeneous areas, or environmental mosaics, the structure and function of landscapes are themselves scale dependent. Hunsaker and Levine (1995) developed methods to characterize landscape attributes that influence water quality at various spatial scales. Understanding how scale, including both data resolution and geographic extent, influences landscape characterization and understanding the way terrestrial processes affect water quality are important for model development and translation of research results from experimental watersheds to management of large drainage basins.

U.S. EPA has a new "watershed protection approach," the U.S. Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) targets large river basins (Gilliom *et al.*, 1995), and some U.S. states such as North Carolina have adopted a "whole basin approach." These activities are discussed in more detail in the next section. Through the comparison of results from monitoring, assessment, and management over time using these different spatial approaches (for example, ecoregion versus watershed), we will be able to determine the value of each approach for management of aquatic resources.

THE ROLE OF FEDERAL AND STATE MONITORING AND ASSESSMENT PROGRAMS. Nonprofit organizations, federal and state programs, and others have been working to establish innovative working relationships and approaches to assess and improve the quality of our nation's waters. Water Quality 2000—a cooperative effort of more than 80 public, private, and nonprofit organizations—involved the development of an integrated national policy for U.S. water quality (WEF, 1992). The impetus for this effort was the fact that while progress has been made in improving the condition of the nation's waters over the past 20 years, the national goal of "fishable and swimmable" waters has not been attained in many areas. The broad objective of the Clean Water Act is to restore and maintain the chemical, physical, and biological integrity of the nation's waters. The final report from Water Quality 2000, *A National Water Agenda for the 21st Century* (WEF, 1992), states that the U.S. has no unified national policy that observes the principles of integrated land and water resource planning and management. Current water policies comprise a patchwork of narrow, often conflicting, objectives; jurisdic-tional conflicts mark both the legislative and executive branches of the federal and some state governments; sound economic principles are often missing from resource allocation decisions; and many sources of water quality impairment remain unaddressed or underaddressed. Water Quality 2000 concluded that a new national water policy is needed to integrate planning and management on a watershed basis. Water Quality 2000 developed 85 specific consensus recommendations, including several related to increasing scientific understanding and improving technologies:

- Initiate detailed, long-term water quality monitoring programs in representative watersheds to advance the science of watershed planning and management;
- Develop and adopt biocriteria and biological monitoring; and
- Increase research and development for restoration of degraded or converted aquatic habitat.

In this section we discuss several federal and state programs that address some of these needs.

Federal Programs. Three federal programs are discussed to illustrate the way that data can be important by providing baseline or background information for state- or site-specific planning and water quality permitting. These programs also represent the current direction toward regional planning and more holistic or ecologically based monitoring and assessment.

U.S. EPA has renewed its effort to focus on hydrologically defined drainage basins—watersheds—rather than on areas arbitrarily defined by political boundaries for water pollution control programs. This watershed protection approach (WPA) is an integrated, holistic strategy for more effectively restor-

ing and protecting aquatic ecosystems (U.S. EPA, 1993b). The WPA is not a new centralized government program that competes with or replaces existing programs, but it is a flexible framework for focusing and integrating current efforts and for exploring innovative methods to achieve maximum efficiency and effect.

U.S. EPA's EMAP is an interagency effort (primarily National Oceanographic and Atmospheric Administration and U.S. Department of Agriculture) to provide long-term monitoring to determine status and trends in ecological resources at regional and national scales (Thornton *et al.*, 1993). The need to establish baseline ecological conditions as a reference for assessing future change has grown more acute with the increasing complexity and scale of environmental issues—for example, biodiversity, sustainability of ecosystems, global atmospheric change. The EMAP should provide data and assessments that will be critical to U.S. EPA's ecological risk assessment effort. The EMAP began addressing ecological resources in eight resource categories: near-coastal ecosystems, inland surface waters, the Great Lakes, wetlands, forests, arid ecosystems, agroecosystems, and landscapes. The conceptual strategy for indicators defines categories of indicators: response, exposure, habitat, and stressor (Hunsaker and Carpenter, 1990). Response indicators are the primary measurement endpoints for EMAP and should quantify the biological conditions of ecosystems by measuring organisms, populations, communities, or ecosystem characteristics. The EMAP seeks to determine spatial and temporal associations between indicators of biological response and indicators of environmental stress and exposure when significant change or unacceptable conditions are identified. Hunsaker (1993) discusses the status of indicator selection, indicator framework, and the linkage between indicator selection and successful assessment. Thornton *et al.* (1994) describes the assessment framework. At this time, EMAP does not have definite descriptions of what biological monitoring data or regional assessments will be like, but there are some preliminary examples from the aquatic resource groups (Macauley *et al.*, 1994; Paulsen *et al.*, 1991; Paulsen and Linthurst, 1994; and Summers *et al.*, 1993). Publications as of 1993 are listed in Lear and Chapman (Eds.) (1994).

The USGS's National Water Quality Assessment (Gilliom *et al.*, 1995, and Hirsch *et al.*, 1988) was funded in 1986 to accomplish the following:

- Provide a nationally consistent description of current water quality conditions for a large part of the nation's water resources;
- Define long-term trends (or lack of trends) in water quality; and
- Identify, describe, and explain, as possible, the major factors that affect observed water quality conditions and trends.

The NAWQA has ongoing and proposed investigations for 60 of the nation's most important river basins and aquifer systems, which are referred to

as *study units*. More than two-thirds of the nation's freshwater use occurs within the 60 study units. The study units include river basins in which the focus of attention is on surface water quality (Figure 5.4) and aquifer systems in which the focus of attention is on groundwater quality. Each study unit investigation will be conducted by a small team of individuals familiar with the river basin, and the study units will be linked together to form a national program with perennial and rotational assessment activities. The program will focus on water quality conditions that are prevalent or large in scale and persistent in time. Biological measurements will be used in the assessment to assist in determining the occurrence and distribution of waters contaminated by fecal material, determining the occurrence and distribution of potentially toxic substances, assessing the relations between the physical and the chemical characteristics of streams and the functional or structural aspects of the biological community, and defining and quantifying biological processes that affect the physical and chemical aspects of water quality.

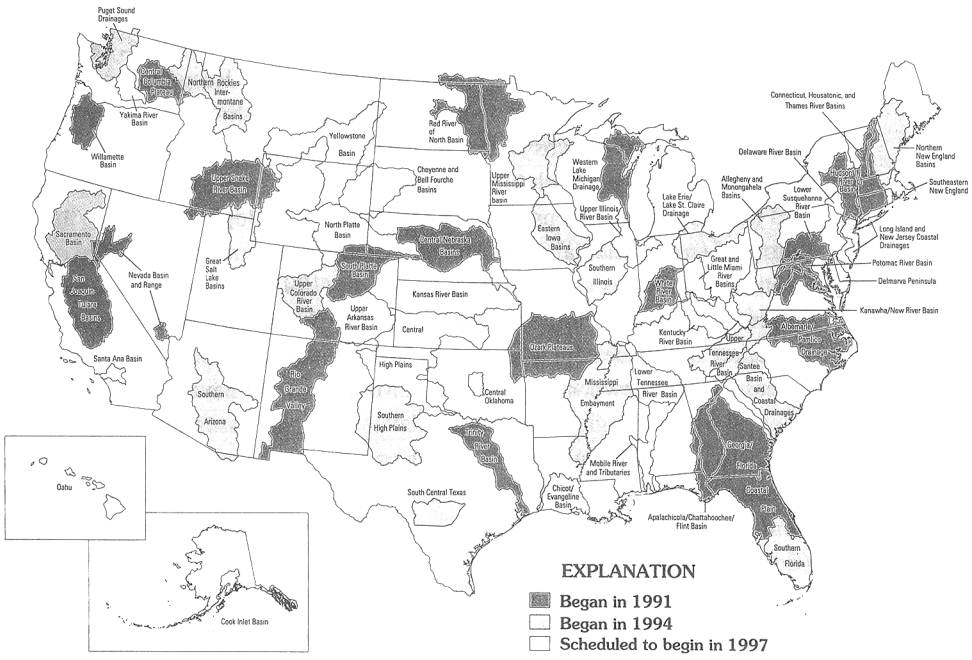


Figure 5.4 Study units of the National Water Quality Assessment (Gilliom *et al.*, 1995)

The NAWQA program builds upon an existing base of water quality studies of the USGS and those of other federal, state, and local agencies. Surface water sampling activities will include fixed-station sampling each month and during high streamflows, synoptic sampling to provide a finer degree of spatial resolution, and studies of selected stream reaches to address certain narrowly focused issues identified during other monitoring. National synthesis of

data analysis, based on aggregation of comparable information obtained from the study units, is a major component of the program. This effort focuses on selected water quality topics using nationally consistent information. Comparative studies will explain differences and similarities in observed water quality conditions among study areas. The first topics addressed by the national synthesis are pesticides, nutrients, volatile organic compounds, and aquatic biology.

State Programs. A few U.S. state programs are briefly described here to give examples of how states can implement the concepts discussed earlier in this chapter. The implementation of whole-basin planning for water quality is described for North Carolina. Some states are establishing biological and water quality criteria based on ecological regions rather than river drainage basins. Results from research in Ohio and Arkansas are discussed to illustrate the correspondence between fish distributions and water quality parameters with ecoregions.

"Current efforts to develop and implement biological criteria are closely related to independent state efforts to apply biological monitoring information to water resource quality assessments" (Southerland and Stribling, 1995). U.S. EPA has published several documents that give guidance on the development of biological criteria (U.S. EPA, 1990), and these publications can provide useful background information on biological monitoring (U.S. EPA, 1991a, 1991b, and 1991c). The first detailed technical guidance document by water body type is available, *Biological Criteria: Technical Guidance for Streams and Small Rivers* (Gibson [Ed.], 1994). Biological criteria provide a mechanism for assessing aquatic life attainment based on actual biological conditions of water bodies and will support state water quality standards. State water quality assessments (Section 305b reports under the Clean Water Act) will include more reporting on biological data. The use of biological criteria in the determination of total maximum daily loads is being explored. Southerland and Stribling (1995) report on the status of state biological assessment programs as of 1994 (Table 5.2). Forty-six U.S. states focus on the benthic macroinvertebrate assemblage as all or part of their sampling programs. Twenty-five of these states also have fish assemblage monitoring programs. Periphyton has received recent attention, with three states using periphyton in monitoring.

At present, few states can characterize even a small fraction of their water resources with biological survey data. Florida, Kentucky, Maine, New York, North Carolina, Ohio, and Wisconsin pioneered the development of bioassessment programs in the 1970s and 1980s. Three states (Ohio, Maine, and Florida) currently have incorporated numeric biological criteria to their water quality standards. In the case of Ohio, these are supported by the most extensive sampling, assessment, and implementation program in the nation (Yoder and Rankin, 1995). North Carolina is applying rigorous numeric biological

Table 5.2 **Status of biological assessment programs and biological criteria programs in the U.S. as of 1994 (reprinted with permission from Southerland, M.T., and Stribling, J.B. [1995] Status of Biological Criteria Development and Implementation. In *Biological Assessment and Criteria, Tools for Water Resource Planning and Decision Making*. W.S. Davis and T.P. Simon [Eds.]. Copyright Lewis Publishers, an imprint of CRC Press, Boca Raton, Fla.)**

	Bioassessment target assemblage			Use of bioassessment as biocriteria			
State	Fish	Benthos	Periphyton	Used in regulations	Used in water resource management	Biocriteria in development	None or unknown
Alabama		✓			✓		
Alaska		✓				✓	
Arkansas	✓	✓			✓		
Arizona		✓				✓	
California		✓				✓[a]	
Colorado		✓				✓	
Connecticut		✓			✓		
Delaware		✓		✓[b]	✓		
District of Columbia		✓				✓	
Florida		✓		✓		✓[a]	
Georgia		✓				✓	
Hawaii							✓
Idaho	✓	✓			✓		
Illinois	✓	✓				✓	
Indiana	✓	✓				✓	
Iowa	✓	✓				✓	
Kansas		✓				✓	
Kentucky	✓	✓	✓		✓		
Louisiana	✓	✓				✓	
Maine		✓		✓			
Maryland		✓				✓	
Massachusetts	✓	✓			✓		
Michigan	✓	✓			✓		
Minnesota	✓	✓			✓		
Mississippi		✓			✓		
Missouri		✓				✓	

Table 5.2 **Status of biological assessment programs and biological criteria programs in the U.S. as of 1994 (reprinted with permission from Southerland, M.T., and Stribling, J.B. [1995] Status of Biological Criteria Development and Implementation. In *Biological Assessment and Criteria, Tools for Water Resource Planning and Decision Making.* W.S. Davis and T.P. Simon [Eds.]. Copyright Lewis Publishers, an imprint of CRC Press, Boca Raton, Fla.) (continued)**

	Bioassessment target assemblage			Use of bioassessment as biocriteria			
State	Fish	Benthos	Periphyton	Used in regulations	Used in water resource management	Biocriteria in development	None or unknown
Montana		✓	✓		✓	✓[c]	
Nebraska	✓	✓			✓		
Nevada							✓
New Hampshire		✓				✓	
New Jersey		✓			✓		
New Mexico		✓				✓	
New York		✓			✓		
North Carolina	✓	✓			✓		
North Dakota	✓					✓	
Ohio	✓	✓		✓			
Oklahoma	✓	✓	✓			✓	
Oregon	✓	✓			✓		
Pennsylvania	✓	✓			✓		
Puerto Rico						✓[a]	
Rhode Island	✓	✓				✓	
South Carolina	✓	✓				✓	
South Dakota							✓
Tennessee	✓	✓				✓	
Texas	✓	✓			✓		
Utah		✓				✓	
Vermont	✓	✓			✓		
Virginia		✓				✓	
Virgin Islands							✓
Washington	✓	✓			✓		
West Virginia	✓	✓				✓	

Table 5.2 **Status of biological assessment programs and biological criteria programs in the U.S. as of 1994 (reprinted with permission from Southerland, M.T., and Stribling, J.B. [1995] Status of Biological Criteria Development and Implementation. In *Biological Assessment and Criteria, Tools for Water Resource Planning and Decision Making.* W.S. Davis and T.P. Simon [Eds.]. Copyright Lewis Publishers, an imprint of CRC Press, Boca Raton, Fla.) (continued)**

	Bioassessment target assemblage			Use of bioassessment as biocriteria			
State	Fish	Benthos	Periphyton	Used in regulations	Used in water resource management	Biocriteria in development	None or unknown
Wisconsin	✓	✓			✓		
Wyoming		✓				✓	

[a] Currently in the process of developing a multimetric approach for assessing community-level effects using benthic macroinvertebrate data.
[b] Has numeric biocriteria (benthos) for estuaries, specifically for regulation of marinas and marina siting.
[c] Currently developing methods for assessment of lakes using periphyton data.

criteria programs to water resource management activities without incorporating them directly to water quality standards. Arkansas and Texas have used biological criteria to assign aquatic life use classifications based on natural conditions appropriate to different regions. Connecticut, Nebraska, New York, and Vermont are using biological criteria to evaluate impairment and support compliance based on existing permits and administrative rules (Bode and Novak, 1995, and Horning *et al.*, 1995). It is envisioned that by the end of the decade, nearly all of the state water resource agencies will have some form of biological monitoring that will aid in determining the effectiveness of best management practices and other mitigation measures (Southerland and Stribling, 1995).

The North Carolina Division of Environmental Management, Water Quality Section, has a whole-basin approach to water quality management. Whole-basin plans should encourage consistent decision making by clearly defining long-term goals and approaches. The target audience for these plans includes policy makers, the general public, and the regulated community. Consistency, together with greater attention to long-range planning, will promote a more equitable distribution of assimilative capacity, explicitly addressing the tradeoffs among pollutant sources (point and nonpoint) and allowances for future growth. North Carolina has defined 17 major river basins, and water quality and aquatic resources will be assessed simultaneously throughout an entire basin. The benefits of whole-basin planning and management are improved efficiency, increased effectiveness (partly because they are based on

ecological principles of watershed management), and consistency and equitability. The Clean Water Act supports whole-basin water quality management under sections 303(e), 303(d), 319, 201, and 208.

The implementation of whole-basin planning and management occurs in phases. Permitting activities and associated routine support activities in North Carolina (such as field sampling, modeling, and waste load allocation calculations) were rescheduled by basin. All National Pollution Discharge Elimination System (NPDES) permit renewals within a basin will occur simultaneously and be repeated at 5-year intervals. The NPDES permit renewal schedule drives the schedule for developing and updating whole-basin management plans. Each whole-basin management plan for North Carolina has eight major sections (Creager and Baker, 1991):

- An introduction, describing the purpose and format of the plan;
- A general basin description;
- An assessment of the current status of water quality and biological communities in the basin;
- An overview of existing pollutant sources and loads;
- Identification of significant water quality concerns and priority issues in the basin;
- Long-range management goals and general management strategy;
- Recommended total maximum daily loads, waste load allocations (for point sources), load allocations (for nonpoint sources), and management actions; and
- Implementation, enforcement, and monitoring plans.

Biological monitoring meshes well with aspects of whole-basin planning and Section 305(b) reporting. Biological information has become integrated to every phase of operations within North Carolina's Water Quality Section. Refined narrative biological standards support the use of bioassessments in evaluating point source and nonpoint source pollution. Biological data support enforcement actions and can form the basis of water quality standards 401 certification for new facilities. Unlike other methods, bioassessments can be used to document in-stream improvements that result from wastewater facility upgrades and the implementation of best management practices (Southerland and Stribling, 1995).

Relationships between ecological regions and fish distribution and/or water quality have been examined for several states (Hughes and Larsen, 1988; Larsen *et al.*, 1986 and 1988; Lyons, 1989; Rohm *et al.*, 1987; and Whittier and Hughes, 1988). The correspondence between fish assemblages and water chemistry data suggests that an ecoregional framework can be a useful context for integrating terrestrial and aquatic systems. Examples of work in Ohio and Arkansas are presented.

Ohio is one of three states that has incorporated numeric biological criteria to water quality regulations. Figure 5.5 shows current biological criteria by ecoregion, and Yoder and Rankin (1995) discuss the development and current status of these criteria in detail. In Ohio, the state was initially divided into four aquatic ecoregions, and water chemistry and fish data were sampled in 46 streams that had watersheds relatively undisturbed by human activities. Monitoring sites were sampled two or three times from July to October of 1983. Patterns in the data were analyzed using a variety of univariate and multivariate methods. There were differences among the fish assemblages that corresponded to the ecoregional pattern (Larsen *et al.*, 1986). In streams of the Huron/Erie Lake Plain, fish assemblages were lowest in species richness and in overall biotic integrity and were dominated by species that are tolerant of sedimentation and turbidity. At the other extreme, in streams of the Western Allegheny Plateau, fish assemblages contained significantly more species, higher biotic integrity, and more species intolerant of sedimentation and turbidity. Fish assemblages in the Eastern Corn Belt Plains and the Erie/Ontario Lake Plain-Interior Plateau were intermediate between these extremes.

Water quality parameters in Ohio were grouped into nutrient richness and ionic strength (Larsen *et al.*, 1988). Water quality results support the fish assemblage results, which indicate that the Western Allegheny Plateau is significantly different from the Huron/Erie Lake Plain. Sites in the Western Allegheny Plateau were concentrated in the area of low nutrient richness and low to intermediate ionic strength. Sites in the Interior Plateau formed a close group with intermediate values for ionic strength and nutrient richness. Sites for the Erie/Ontario Lake Plain were more scattered and encompassed those of the Interior Plateau. Sites in the Huron/Erie Lake Plain and the Eastern Corn Belt Plains were similar along the ionic strength axis but separated somewhat along the nutrient richness axis. Larsen *et al.* (1988) conclude that land classification systems such as Omernik's ecoregions can be used to characterize attainable stream water quality. They define attainable water quality as that found in streams and watersheds that are both representative of their ecoregions and least disturbed by human activities.

Criteria for the index of biotic integrity (IBI), invertebrate community index, and modified index of well-being have been developed for different site types within each ecoregion. These numeric indices provide specific quantitative measures that must be met to attain the tiered aquatic life uses stipulated in Ohio's water quality standards. The information provided by Ohio's biological criteria program has been useful for virtually all regulatory, resource protection, and monitoring and reporting programs pertaining to surface waters. Barbour *et al.* (1995) discuss the use of multimetric indicators such as the IBI in more detail, while others (Suter, 1993) caution about their use.

Arkansas was divided into six aquatic ecoregions, and fish, physical habitat, and water quality were sampled in 22 streams (Rohm *et al.*, 1987). Patterns developed separately from fish, physical habitat, and water quality data

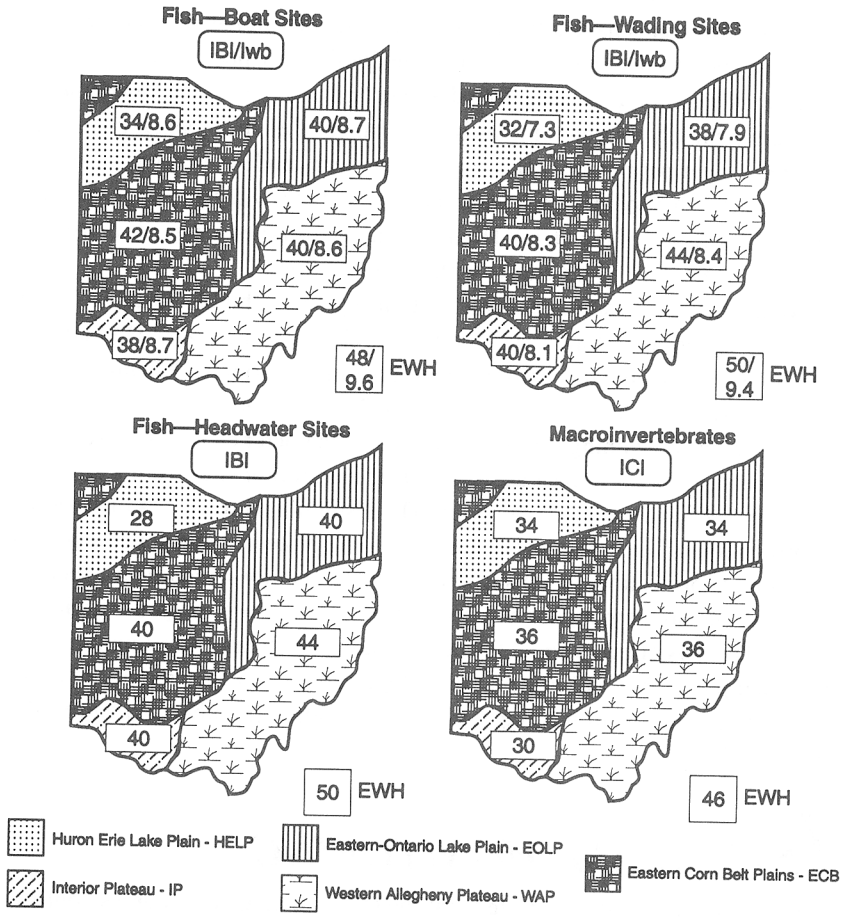


Figure 5.5 **Biological criteria in the Ohio water quality standards for warmwater habitat use and exceptional warmwater habitat (EWH) use designations arranged by biological index, site type for fish, and ecoregion; the EWH criteria for each index and site type are located in the boxes outside of each map (IBI = index of biotic integrity; ICI = invertebrate community index; and Iwb = modified index of well-being) (Yoder and Rankin, 1995)**

sets were relatively concordant. The most distinctive ecoregional patterns were observed in fish species presence/absence and abundances in different ecoregions. The characteristic species of the Mississippi Floodplain region are typically found in clear yet soft- and muddy-bottomed waters distinguished by little or no current and abundant aquatic vegetation. The characteristic fishes of the South Central Plains include species that typically occur over hard sandy or gravelly bottoms in slight permanent currents and seem to be less dependent on vegetation than the Mississippi Floodplain fishes. Habitat for

fish in the Arkansas Valley is gravelly substrate and riffles. The sites of the Ouachita Mountains have a fair amount of variability in fish assemblages and chemical and physical habitat. Most of the dominant fishes of the Boston Mountains are similar to those found at one or more sites in the Ouachitas. They are typically tolerant of a fairly wide range of environmental conditions but prefer clear permanently flowing streams with moderate currents (can inhabit sluggish pool and backwater habitats and can withstand sporadic turbidity). The fishes of the Ozark Highlands have restricted ranges and are found in small, cool, clear creeks with moderate flows.

Water chemistry parameters defined four regions. A highly variable yet distinct Mississippi Floodplain, the South Central Plains, the Ozark Highlands, and the Boston Mountains combined with the more variable Ouachita Mountains and Arkansas Valley. Rohm *et al.* (1987) concluded that the identification of regions containing streams with similar character can assist in selecting reference streams for impact assessments or monitoring sites from which results may be extrapolated regionally. The result of this work was the specific identification of the biological community to be protected and a methodology to ensure its protection. Arkansas now has water quality standards for specific locations that are both higher and lower than the dissolved oxygen criterion of 5 ppm. This has allowed small towns on headwater streams to begin building treatment plants that will protect the biological community at a reduced cost compared to the cost of meeting a statewide standard (Southerland and Stribling, 1995).

IN-STREAM MONITORING

Hughes and Noss (1992) reported that the status of aquatic resources continues to deteriorate. Monitoring programs have often failed for lack of a well-defined purpose (Perry *et al.*, 1987, and Segar and Stamman, 1986), and components such as identification of what will be measured (endpoints/indicators) and how these measurements will be analyzed must be appropriately defined for the purpose and scale of the program (Hunsaker, 1993, and NRC, 1990). Hughes (1995) suggests that inadequate data interpretation may explain why it is difficult to set policy or make decisions based on monitoring results. Hughes (1995) and Davis and Simon (1995)—and the direction set by national and state programs such as EMAP (Messer *et al.*, 1991) and those of Ohio and North Carolina—provide overviews and substantial guidance on the way to use biological monitoring effectively in water quality programs. Critical issues for biological monitoring include selection and use of reference sites, relationships between laboratory testing and ambient monitoring, and the need for data on chronic and/or episodic stresses.

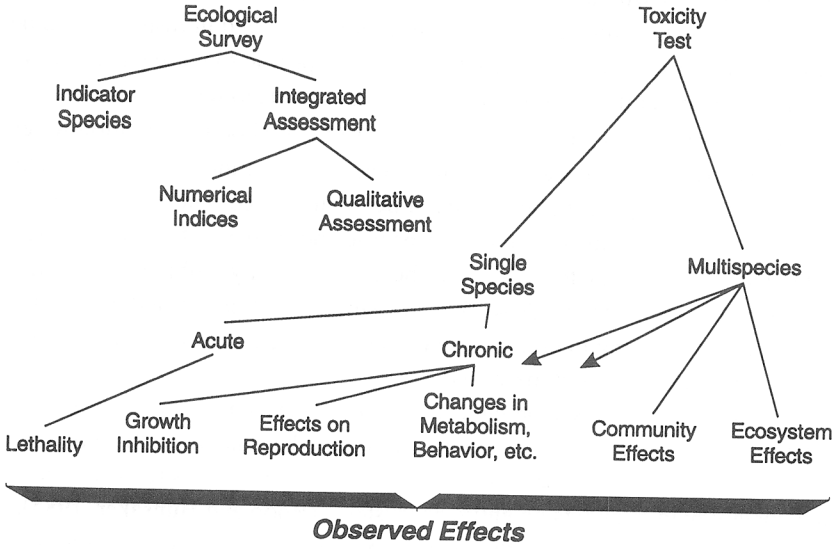


Figure 5.6 **Classification of biomonitoring methods (Roop and Hunsaker, 1985)**

RELATIONSHIPS AMONG LABORATORY TESTING, MICROCOSM AND MESOCOSM TESTING, AND IN-STREAM TESTING AND MONITORING. Biomonitoring methods can be classified into two categories: ecosystem surveys and toxicity testing (Figure 5.6). Ecological surveys may use indicator species, assessments based on the composition of biological communities, numerical diversity and indices, or all of these. By making comparisons between affected and control areas, ecological surveys can indicate the condition of a water body exposed to pollutant loadings. Such an approach is often referred to as *biocriteria* (Davis and Simon [Eds.], 1995). Although ecological surveys provide an assessment of water quality that integrates the effects of exposure over time, the expense of ecological surveys and the length of time required to perform them are often mentioned as disadvantages (Roop and Hunsaker, 1985). However, Yoder and Rankin (1995) provide quantitative data based on their experience in Ohio to counter this impression. They compare the cost of macroinvertebrate community and fish community evaluations with chemical/physical grab sampling and acute and acute/chronic bioassay tests.

Toxicity testing is currently the biomonitoring method most likely to be used in permitting for toxic effluent. In 1984, when U.S. EPA issued its policy on biomonitoring, the agency believed that aquatic toxicity testing methods had matured sufficiently to be used for permitting. However, additional research on several issues now, as then, remains useful. One such issue is the correlation of effluent toxicity measurements to actual in-stream impact. Single-species tests are essential for measuring such things as lethality,

alterations of growth rate, and fecundity; however, basic ecosystem properties, such as nutrient cycling, mineralization, energy flow, and intraspecies competition, must be studied by toxicity tests that are specific to these phenomena. Factors that affect the translation of laboratory results to field conditions include the physical-chemical characteristics of the laboratory dilution water as compared to ambient water; chemical reactivity; continuous versus variable exposure; prior exposure phenomena of debilitation, acclimation, and selection; disease state; nutrition; and intraspecific and interspecific competition and behavior. These factors also contribute to variability within toxicity methods. Another issue that needs work is the validation of endpoints selected for biological monitoring to predict deleterious ecosystem effects (temperature, flows, sedimentation, and toxicants).

Ecotoxicology is based on the concept that ecosystems have emergent properties derived from the system as a whole, rather than from the individual parts. Therefore, many researchers believe that single-species tests cannot adequately predict effects on entire ecosystems (Roop and Hunsaker, 1985), and Cairns (1988) states that the regulation of toxicants should be based on multispecies testing rather than single-species testing. He also suggests that presently used methodology is accepted because of it origins rather than scientific suitability.

Cairns (1990) outlines three steps that are necessary to establish a sound biological monitoring program, and they are paraphrased here:

- Establishing baseline conditions for the community or ecosystem (that is, its structural and functional characteristics, including species composition).
- Developing a conceptual model that determines which attributes are most likely to be first affected as a consequence of anthropogenic perturbations or natural cyclical events. This model can be predictive or retrospective.
- Validating or confirming these predictions in the natural system in question or a surrogate thereof (for example, a microcosm, mesocosm, or field enclosure).

Information should be gathered on the natural variability of attributes of interest, for example, seasonal and other cyclic variation. Whenever possible, standard methods should be used; however, these are still not available for a variety of ecosystems. It is ideal to have information on baseline conditions before a change or development (for example, construction of a dam or new wastewater treatment facility). However, if the sources of perturbation are already in place, reference areas can be selected.

Biological criteria can provide an appropriate reference for monitoring and assessment and can establish statistical and ecological (practical) significance (Davis and Simon [Eds.], 1995). Sometimes there is a question of whether sta-

tistically significant differences are actually ecologically significant because of inherent natural variations of populations and possible improper sample replication (McBride *et al.*, 1993). Confounding effects from natural variability of populations are reduced by using an index period for sampling, applying consistent methodology, and relying on community measures reflecting ecologically important attributes (for example, trophic structure and taxa richness) (Davis and Simon [Eds.], 1995, and Hughes, 1995). Although successful uses of ambient biological monitoring and criteria exist, there is often misunderstanding and concern regarding the application of biological assessments. Davis (1995) briefly reviews how far numeric biological assessment and criteria development has progressed, including a discussion of the Saprobien system, diversity indices, Beck's biotic index, macroinvertebrate indices, and multiple metric indices such as the index of biotic integrity.

REFERENCE SITES AND BIOLOGICAL CRITERIA. Principles for successful development and implementation of numeric biological criteria are based on developing a reference condition (Hughes, 1995) from a regional framework (Omernik, 1995), which may include a multiple metric characterization of the aquatic community (Barbour *et al.*, 1995) and a habitat evaluation (Rankin, 1995). The need for an ecoregional/reference site framework to facilitate the development of biological criteria was recognized in the late 1970s (Warren, 1979) and was related to the growing awareness that biota, physical habitat, and toxicity had to be considered with water chemistry for effective management of aquatic ecosystems. Reference sites characterize the *attainable conditions* for a region rather than *pristine* conditions (absence of humans or of pre-European settlement). Candidate stream sites must be relatively undisturbed yet representative of the ecological region they occupy (Hughes, 1995, and Hughes *et al.*, 1986). An initial selection of reference sites is typically possible using 1:100 000-scale topographic maps, and state water resource managers and regional biologists are aware of streams of interest. State and regional experts should be consulted regarding the minimum number of sites necessary for characterization, which is typically a function of the size and complexity of the subregion. For some small or homogeneous regions, the point of diminishing returns may be reached with a number of five or six. Reference sites representing least-disturbed ecosystem conditions are a moving target of which humans and natural processes are a part, and they are relative to their region. In the Boston Mountains ecoregion (in Arkansas and Oklahoma), reference sites comprise streams having watersheds without point sources, with little grazing activity, and with a relative lack of recent logging activity and road building. Thus, these streams fit most people's perceptions of a high-quality stream condition. In the Huron/Erie Lake Plain Ecoregion (in Ohio, Indiana, and Michigan), most streams are completely in cultivated agriculture. Although the quality of the reference streams reflects the range of best

attainable conditions given current land-use patterns in the region, this does not imply that quality cannot be improved (Omernik, 1995).

Federal regulation requires states to develop and apply biological criteria (U.S. EPA, 1990), and EMAP's surface waters program should assess a water body's biological condition as acceptable or unacceptable. Both of these activities require a reference condition, and Hughes (1995) provides excellent direction by explaining the purpose and objective of determining a reference condition for populations of aquatic ecosystems, examining six approaches for determining a reference condition, describing the process for selecting regional reference sites, and proposing a way to determine acceptable and unacceptable quantitative scores from reference condition data.

To set reference conditions, water bodies have to be classified by factors such as type and size. Hughes (1995) presents six approaches for determining the reference condition for biological criteria:

- Regional reference sites,
- Historical data,
- Paleoecological data,
- Experimental laboratory data,
- Quantitative models, and
- Professional judgment.

These approaches should not be considered mutually exclusive and can be used in a synergistic fashion. For example, Hughes (1995) and Cairns *et al.* (1993) recommend interpreting information from regional reference sites and historical data using linear models and professional judgment. Paleolimnological data should be considered for natural lakes and some wetlands. Laboratory toxicity testing data can be used to screen out sites selected by other means or improve model predictions. Well-known relationships between test species and specific toxicants, temperatures, nutrients, and dissolved oxygen concentrations indicate stressor levels that are harmful to aquatic communities and can be useful in selecting reference conditions.

Hughes (1995) lists the following eight steps for selecting regional reference sites:

1. Define areas of interest on maps;
2. Define water body types, sizes, and classes of interest;
3. Delineate candidate reference catchments;
4. Conduct aerial or photographic evaluation;
5. Conduct field reconnaissance;
6. Subjectively evaluate quality of candidate reference sites;
7. Determine number of reference sites desired; and
8. Evaluate biological condition of candidate reference sites.

Although the preceding eight steps are listed in order of recommended occurrence, the process should be iterative and not linear. The actual number of regional reference sites needed is a function of regional variability and size, the desired level of detectable change, resources, and study objectives. Three is a commonly used minimum number for any particular water body class and size in a homogeneous region (Hughes, 1995). Twenty randomly selected sites is the minimum number recommended by survey statisticians; however, this number depends on desired detection levels. At least two assemblages should be surveyed as the final step in selecting regional reference sites. This is often the only way to determine whether species (especially fish) were introduced or learn if some unknown stressor is acting on a system.

Hughes (1995) summarizes the problematic aspects of determining whether an ecosystem is in acceptable or unacceptable condition. There are encouraging examples of success in Ohio (Yoder and Rankin, 1995) and Maine (Courtemanch, 1995). Hughes (1995) compares the process to that of toxicity tests: "Initially, aquatic toxicity tests were viewed with considerable skepticism over their value as indicators of anything; now they are broadly accepted, regularly used, standardized, and often diagnostic, despite the lack of any ecological significance to an LC-50."

FUTURE DIRECTIONS: MANAGING AQUATIC ECOSYSTEMS FOR EPISODIC TOXICITY. Managing aquatic ecosystems at the watershed/drainage basin level has long been an objective of ecotoxicologists. Watershed management by nature dictates that loadings to an aquatic ecosystem and their sources be understood. It is equally important that the effect of these loadings on the system be understood. The rapid evolution of computers, communications links, geographical information systems, and remote sensing has contributed to advancing the tools necessary to achieve this objective. Continuous-monitoring gauging stations that record stage height, flow, and selected physical-chemical factors already exist through agencies such as USGS and the Tennessee Valley Authority. However, it is impossible to understand and manage an aquatic system without understanding its biology. It is also probably unrealistic to believe that one can effectively manage an aquatic system without having some real-time or near-real-time measures of its biological status.

The implementation of the water-quality-based approach to toxicant control (U.S. EPA, 1984) through permitted point source discharges from municipal and industrial facilities (NPDES permits) should result in increased control of water-column-borne toxicants from traditional point sources. Current accelerated efforts to understand and control the role of sediment as a sink and potential source for toxicants will further our understanding of watershed dynamics. Nonpoint source pollution or point source pollution from stormwater conveyances in metropolitan areas presents some challenging ecotoxicological problems because of its episodic nature.

Cairns and Mount (1990) stated, "No instrument has ever been devised that can measure toxicity! Chemical concentrations can be measured but only living material can be used to measure toxicity." Renewed interest in real-time or near-real-time biomonitoring methods has been prompted by published research on aquatic systems (Dickson *et al.*, 1991 and 1992, and Waller *et al.*, 1991) and years of experience with stormwater runoff evaluations, water quality assessments, community structure and function analyses, and ambient toxicity testing. Three of the most important factors in dealing with episodic toxicity events are the following: Is a toxicity event occurring? What is the cause of the toxicity? What is the source of the toxicant(s)?

Traditional effluent toxicity tests (U.S. EPA, 1991d) can and have been used to determine if a sample taken during an episodic event is toxic. Seven-day, short-term chronic toxicity tests performed in a manner consistent with effluent toxicity procedures using the fish *Pimephales promelas* and the cladoceran *Ceriodaphnia dubia* can answer this question. However, these tests probably only infrequently mimic the doses observed by resident aquatic organisms exposed to episodic events. Furthermore, as is true with effluent tests, these tests only reveal whether a sample is toxic after the test is under way or completed. Many episodic events associated with stormwater runoff are not toxic; therefore, a lot of effort is expended evaluating nontoxic samples. A significant redirection of effort could be achieved if one could confidently determine whether an ongoing event was toxic (U.S. EPA, 1991e, 1992b, 1993c, and 1993d). Aquatic animals have been shown to induce bioelectric signals to surrounding water that can be recorded as rhythmic analog signals representative of specific movement activities (for example, gill beats and heart rates). In addition, gape measurements (width of shell opening) have been used with clams and mussels to determine the condition of such organisms. Using appropriate statistical techniques and accompanying electronics, changes in bioelectric action responses can be detected and processed and have been used in detecting water-quality-induced stress in aquatic organisms. The concept of using bioelectric action potentials to monitor the well-being of aquatic organisms is not new (Cairns *et al.*, 1970). However, early attempts were hampered by the level of technology available at the time they were conceived.

In the early 1970s, Cairns *et al.* (1970, 1972, and 1975) proposed a biological monitoring system for a watershed drainage that would provide an early warning of water pollution. In addition to stream surveys, Cairns and his coworkers described a unique system for automatically recording fish breathing and swimming activities in response to developing toxicity in effluent and ambient receiving water. Support for this approach was based on the observation that a variety of aquatic animals produce discrete bioelectric action potentials as a result of specific physiological activities. For example, the intimate contact between the water and respiratory surfaces of fish gills (and, presumably, gills of other aquatic organisms) makes these organs particularly suscep-

tible to toxic materials (Morgan *et al.*, 1988b). Changes in rhythmic patterns can be detected electronically (Morgan *et al.*, 1981).

Since the early 1970s, numerous attempts have been made to use remotely sensed bioelectric action potentials to detect adverse conditions (Morgan *et al.*, 1981, 1986, 1987a, 1987b, 1988a, and 1989). Morgan *et al.* (1986) have used microprocessor-based monitors to measure fish breathing rates, in which the biosensing units were interfaced with a U.S. National Aeronautics and Space Administration remote data collection platform on the National Oceanic and Atmospheric Administration geostationary operational environmental satellite (GOES) for retrieval and transmission of data. Four times each day, data collected at the remote data collection platform were transmitted to the overpassing GOES.

Caspers (1988), Matthias and Puzicha (1990), and Sloff *et al.* (1983) have also been involved in the use of remotely sensed bioelectric action potentials as a means of detecting adverse environmental conditions for aquatic organisms. Specific biomonitors evaluated include the rheotaxis of fish (Juhnke and Besch, 1971), the respiration of rainbow trout (Sloff, 1979), and the electric field alteration of the tropical fish *Gnathonemus petersi* among others (Geller, 1984). Another European study used the mussel *Dreissena polymorpha* as a biological monitor (Borcherding, 1992).

One can envision a network of watershed-gauging stations in which not only sophisticated measures of physical and chemical parameters are continuously monitored and transmitted by satellite to some remote site for real-time or near-real-time analysis and interpretation, but sites that are also outfitted with a suite of biological sensors, including a variety of fish, insects, and clams (Figure 5.7). These sites would be linked to samplers at and between the sites that would be activated by biomonitored organisms as they respond to adverse conditions. Samples collected under these sets of conditions would be confirmed for toxicity using another group of organisms like *Pimephales promelas* and *Ceriodaphnia dubia* or their saltwater equivalents. The toxic samples could be subjected to toxicity identification procedures to isolate the causative toxicant(s). The entire watershed management system would be connected by satellite or other electronic telecommunications network to a regional control center. A framework for biomonitoring like the one described here is not intended to prevent toxicity events before they occur but to be more effective than laboratory toxicity testing at detecting episodic events. Such monitoring would help prevent future toxicity events from occurring by identifying the toxicants associated with episodic events, locating their sources, and undertaking corrective measures. They would also allow a more rapid response to episodic toxicity events.

Toxicity identification evaluation procedures (U.S. EPA 1991e, 1992b, 1993c, and 1993d) are tools to help identify the causes of toxicity. These procedures, which use physical and chemical separation techniques to help sort through the various classes of potential toxicants, have been used to identify

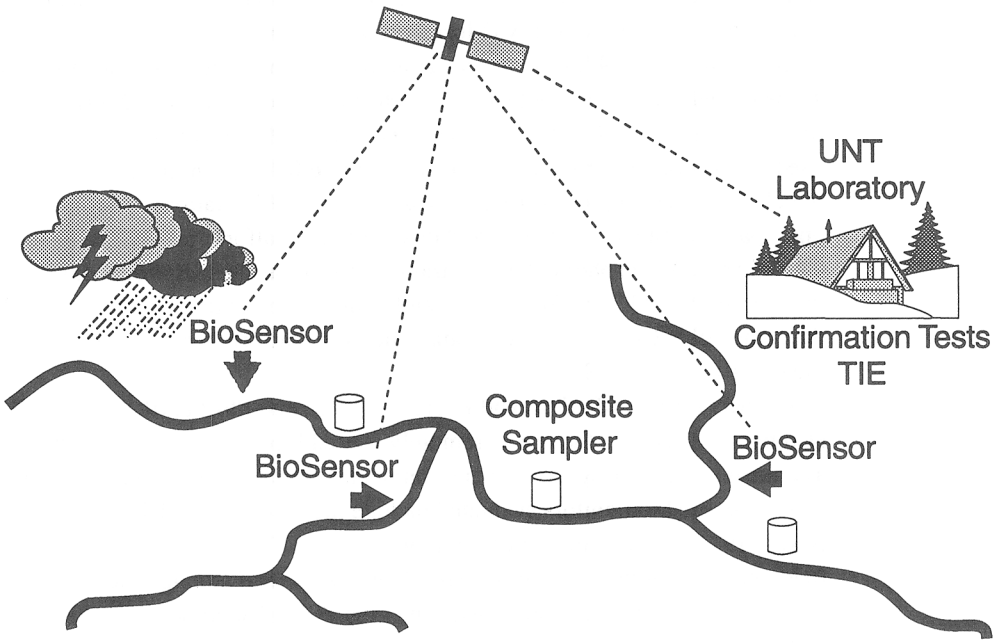


Figure 5.7 Diagram of the use of biosensors in watershed management

the causes of toxicity in complex effluent samples (see Chapter 7). The methods have also been applied to stormwater samples.

SUMMARY AND CONCLUSIONS

Karr (1995) states that we have managed water resources as if the biological systems associated were incidental to society. He lists five realities that point to the need for change: the biological components of water resources are in steep decline; the cause of the decline is broader than simple chemical contamination, the primary focus of conventional water quality programs; the legal and regulatory framework in place today cannot respond in a timely manner to these two realities; long-term success in protecting water resources requires careful thought about assessment endpoints, including development of biological criteria; and the quantitative expectations for attributes that constitute biological health or the integrity of a water resource system vary geographically. Biological monitoring is and will be critical in planning and developing regulatory actions to protect water quality and aquatic ecosystems.

Perhaps the most important message from this chapter should be that the concepts of watershed management, ecosystem management, biological monitoring, biological criteria, toxicity testing, NPDES permits, and ecological risk assessment are not entirely disjunct; they should play synergistic roles in

moving toward the goal of protection and use of water bodies and aquatic ecosystems.

While biological monitoring focuses on the use of biological organisms as indicators for the process, often information on chemical and physical characteristics are necessary to understand or suggest a cause for a trend or change. Thus, biological monitoring, biological criteria, and/or toxicity testing may provide much of the information necessary for watershed or ecosystem management, but such comprehensive management will likely require other data such as land cover and soils at appropriate spatial and temporal scales. The concept of scale is important to ensure that analyses are highlighting issues rather than obscuring them. We have also tried to show how ecological theory can play an important part in the development of applied techniques. The river continuum and landscape ecology concepts are examples. Focusing on the heterogeneity and pattern of the landscape in a watershed or region may be more difficult than forcing it into a homogeneous paradigm, but this approach should ultimately be rewarding for management.

As we stated at the beginning of this chapter, the purpose of the Clean Water Act is to protect both human health and the condition of biological organisms that live in surface waters. Integrated assessment will require biomonitoring data and quantitative assessment techniques as discussed in this chapter. Activities like Water Quality 2000 strive to create an atmosphere where integrated assessment can progress. Monitoring can occur for regulatory purposes or for general assessment purposes. Assessment needs should dictate monitoring specifications; however, often the assessment function lags behind monitoring in both time and research effort (NRC, 1990). While assessment and management should be separate activities so that assessment is not biased by management's desires (note the distinction between risk assessment and risk management in Figure 5.1), the two functions must be interactive to be effective. Biological monitoring has an important role to play in assessing and protecting the desired condition for aquatic ecosystem designated use.

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Chapter 6
Toxicity Reduction Evaluations

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INTRODUCTION

Section 101(a)(3) of the Clean Water Act stipulates a clear national policy that prohibits the "discharge of toxic pollutants in toxic amounts." Where periodic monitoring indicates the continuing presence of unacceptable levels of toxicity in a permitted discharge, regulatory authorities (state or federal) require implementation of a plan to identify the causative factors contributing to the problem and reduce or eliminate those factors. This effort is referred to as a *toxicity reduction evaluation* (TRE).

Scenarios that trigger TREs vary from state to state. Each National Pollutant Discharge Elimination System (NPDES) permittee should, therefore, carefully review its permit regarding TRE triggers.

This chapter will outline some of the concepts and approaches associated with identifying and reducing wastewater toxicants. While the chapter provides common approaches used for industrial and municipal toxicity reduction efforts, it is important to note that there is no single method or approach that can be applied universally. In addition to obvious differences in wastewater complexity, volume, and makeup, dischargers differ in their toxicity identification or reduction objectives. As a result, toxicity identification and reduction efforts can range from simple chemical substitution adjustments in operations to a fairly complex effort requiring input from process and treatment engineers, chemists, toxicologists, treatability specialists, waste minimization/pollution prevention specialists, discharge modelers, and others.

In conducting TREs, it is important to remember that there may be more than one approach to resolving a facility's effluent toxicity problem. The objective is to select the course of action that enables a permittee to meet present, and possibly future, permit requirements cost effectively.

While there is no such thing as a typical TRE, most TREs will include the following five steps:

- Facility operations review,
- Toxicity identification evaluations (TIEs),
- Toxicity source evaluation,
- Toxicity control selection, and
- Toxicity control implementation.

FACILITY OPERATIONS REVIEW

Reducing toxicity in a wastewater stream begins with a careful analysis of in-plant processes that contribute to the wastewater stream. For industrial dischargers, this does not necessarily mean an immediate chemistry scan of contributing process streams but, more important, a review of the entire operation from in-plant manufacturing processes and general housekeeping to treatment plant performance. The same holds true for wastewater treatment plants (WWTPs); an initial analysis or review of existing data, such as a discharge monitoring report (DMR), is often more useful than an immediate priority pollutant chemistry scan, which may be considered the proverbial needle-in-the-haystack approach. Wastewater typically is composed of multiple compounds acting in concert. The unpredictability of such combinations is the principal reason why whole effluent toxicity testing has been incorporated to NPDES permits. A simple analysis for specific compounds will often fall short of predicting toxicity levels or pinpointing the source of the problem.

The first step in reducing toxicity is to conduct a thorough review of historical data. These data can be found in NPDES DMRs, bioassay testing reports, chemical use inventory records and material safety data sheets, facility maintenance records, WWTP operating records, and any other appropriate data

sources that may provide some background for analysis of trends. Questions specific to plant operations include the following: Is there anything different today versus in the past when little if any toxicity was exhibited? Has the product line been changed? Are there new raw products or constituents in the fabrication or manufacturing processes? Table 6.1 provides a more comprehensive list of data/information sources (Fava *et al.*, 1986).

Table 6.1 Checklist of facility-specific data (Fava *et al.*, 1986)

I. Chemicals used
   - A. Amounts
   - B. Material safety data sheets
   - C. Process in which chemicals are used
   - D. Aquatic toxicity/biodegradability information on all chemicals used and their breakdown products

II. Engineering drawings of facilities
   - A. All floor and process drains with schematics
   - B. Potable and wastewater line locations
   - C. Steam line, boiler locations, cooling tower locations
   - D. Wastewater treatment plant schematic
   - E. Production flow chart and line schematic

III. Facility records
   - A. Water usage, water bills
   - B. National Pollutant Discharge Elimination System or monitoring reports for 24 months
   - C. Wastewater treatment plant quality assurance data reports
   - D. Wastewater treatment plant operator interview
   - E. Wastewater treatment plant flow recorder records
   - F. Complete toxicity test history
   - G. National Pollutant Discharge Elimination System (or equivalent) permit

In general, an evaluation is undertaken not necessarily to identify the causative toxicant(s), but to determine whether there are any changes to facility operating activities that could be modified or changed to reduce or eliminate toxicity. The objective may not be to identify the toxicant(s) but simply to remove it or render it harmless in a cost-effective manner. An industrial or municipal WWTP may evaluate toxicity trends based on the addition of new industrial influent or operational changes of existing users.

The initial approach outlined above is enhanced by an extensive database. In addition to data from reports or records, information gathered from in-house process engineers, maintenance personnel, treatment plant operators, and other appropriate staff can be valuable in solving the puzzle as to the cause and subsequent solution to a wastewater toxicity problem. In-house "brainstorming" can be useful when the incidence of toxicity is a fairly new occurrence. Operational questions comparing operations before and after the onset of toxicity are particularly useful.

While one should be cautious using a simple chemical scan approach to address wastewater toxicity, there are some exceptions. If a known toxicant, such as ammonia, is present at elevated concentrations from time to time, it is advisable to check for its presence and verify reduction or elimination of toxicity following its removal. Again, the more knowledge one has of the natural characteristics and makeup of the wastewater stream in question, the better the chances of a quick and less costly resolution of the problem. Figure 6.1 provides a flow diagram of a conventional chemical approach to identifying and reducing toxicity. Although this approach seems simple and straightforward, it is not the best tool in most cases. Complex wastewater characterized by multiple unknowns requires a more thorough toxicity-based approach to identifying and reducing toxicity.

TOXICITY IDENTIFICATION EVALUATIONS

A TIE is a key tool in TREs. Toxicity identification evaluations systematically attempt to characterize, identify, and confirm the causative agents of whole effluent toxicity. Toxicity identification evaluations are interdisciplinary in nature and may require the expertise of chemical and wastewater engineers, analytical chemists, and toxicologists. It is important to remember that identification of the actual toxicant(s) may not always be possible from a cost or technology standpoint. It may be possible, however, to identify the class of compounds that are the cause of toxicity and then develop a strategy to reduce that class of wastewater constituents.

A typical TIE scheme involves subjecting wastewater aliquots or samples from suspect waste streams to a series of characterization tests. Each characterization test is designed to alter or render biologically unavailable a group of potentially toxic compound(s) such as oxidants, cationic metals, volatiles, nonpolar organics, or metal chelates. Aquatic toxicity tests are then conducted on the wastewater aliquots before and after manipulation to reveal whether the treatment is successful in reducing toxicity and provide information on the nature of the toxicant(s). After a consistent pattern is observed in repeated TIEs, this is followed by analytical chemistry procedures to identify specific toxicants from the group(s) characterized in the previous step. The final step

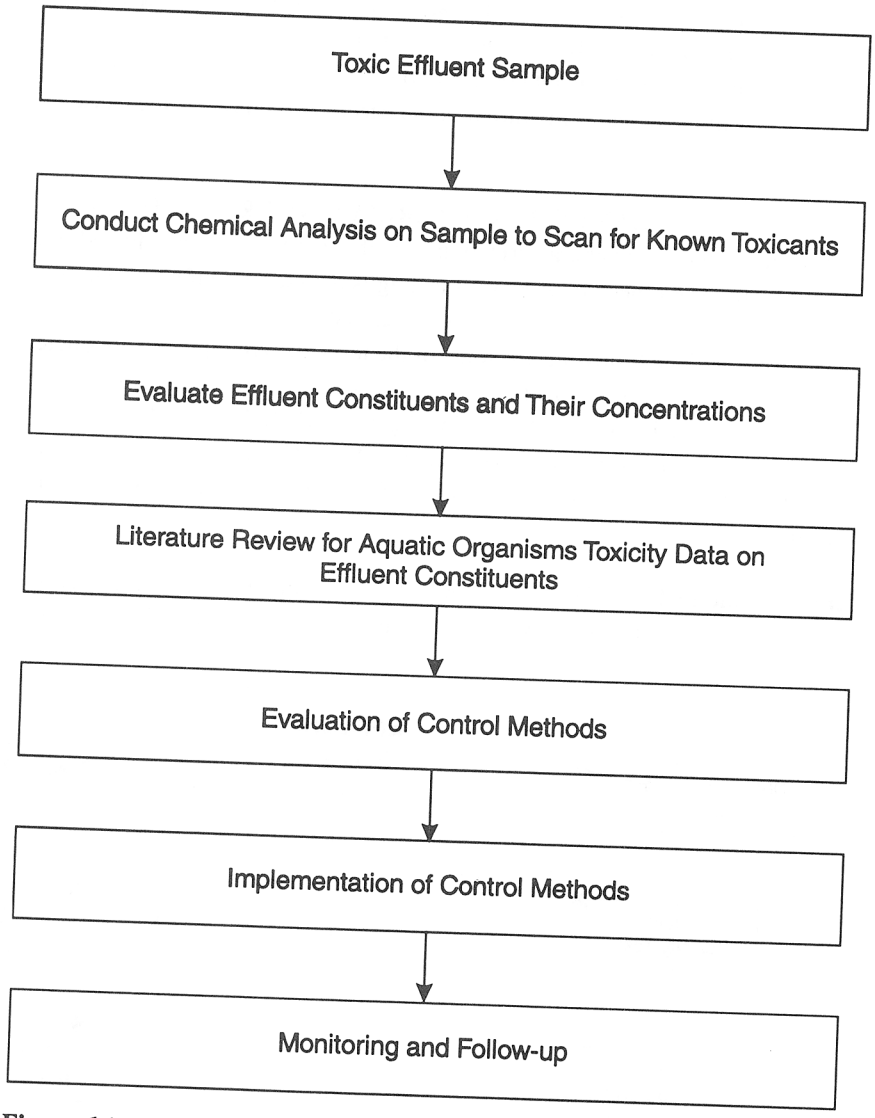


Figure 6.1 **Conventional chemical-based approach to a toxicity reduction evaluation**

in a TIE scheme consists of a group of steps intended to confirm the identity of the suspected toxicants and establish the true cause of toxicity.

There are three principal phases of TIEs:

- Phase I TIE, toxicant characterization, involves manipulating the wastewater to determine if compounds with a common physical or chemical characteristic are responsible for the toxicity of the wastewater;

- Phase II TIE, toxicant identification, attempts to identify the exact chemical composition of the toxicant; and
- Phase III TIE, toxicant confirmation, includes methods that confirm that the toxicant(s) identified in the earlier two phases is the cause of effluent toxicity.

PHASE I—TOXICANT CHARACTERIZATION PROCEDURES. Below are some of the characterization tests that are typically conducted in a Phase I TIE. The objective of Phase I testing is to determine if compounds with a common physical or chemical characteristic are responsible for wastewater toxicity. The following are some of the tests that are typically conducted as part of Phase I characterization. These procedures are discussed in greater detail in U.S. Environmental Protection Agency's (U.S. EPA's) *Methods for Aquatic Toxicity Identification Evaluations: Phase II: Toxicity Characterization Procedures* (1991). A representation of these bench-scale tests is shown in Figure 6.2.

Initial Toxicity Test. This test is conducted as a simple short-term measure of toxicity on an aliquot of a wastewater sample that is intended for Phase I

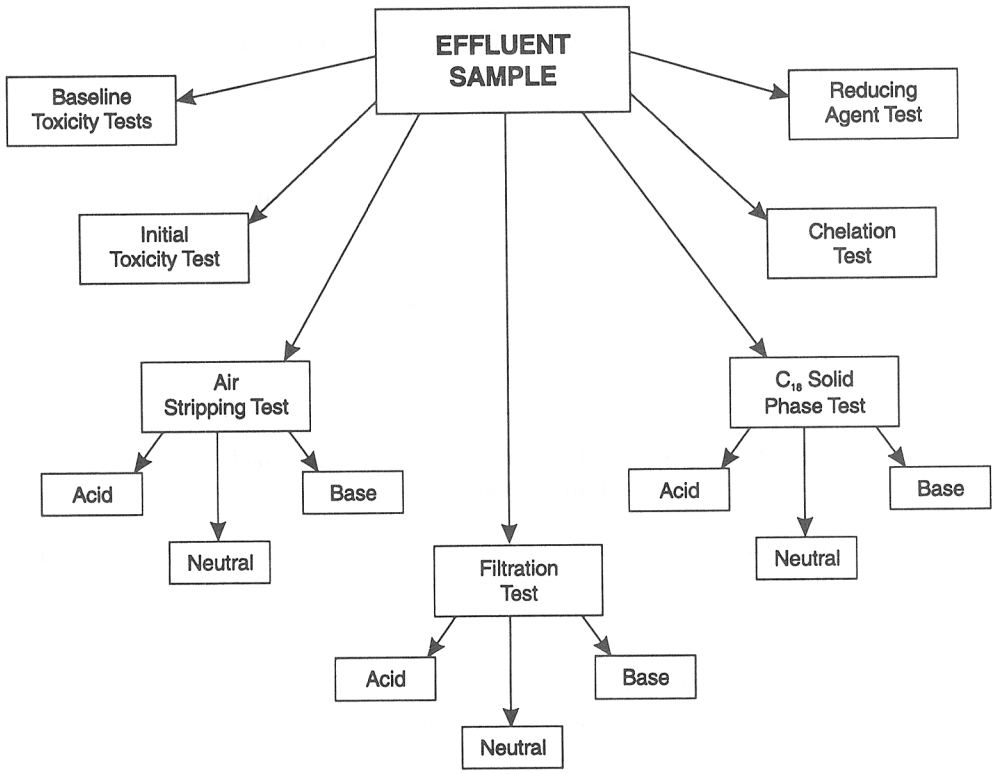


Figure 6.2 Phase I—toxicant characterization tests

characterizations. The purpose of this test is to establish whether the sample is toxic to the selected species. If the test does not show any toxicity, no further characterization is attempted on this sample.

Baseline Test. The baseline test is the focal point of the Phase I portion of the TIE. The baseline test uses unmanipulated wastewater. Survival of organisms in this treatment is compared to all posttreatment bioassay tests. If toxicity (expressed in terms of the concentration of a test material that causes death of a specified percentage of a population, typically expressed as the median or 50% level [LC_{50}]) is decreased in any of the characterization tests as compared to the baseline tests, then that group of compounds would be the suspected causative agents of toxicity in that sample.

For example, the air stripping test is conducted similar to the baseline test, except that wastewater is aerated before introduction of the organisms. If wastewater toxicity is decreased (that is, LC_{50} is higher) in the wastewater that received air, then it can be theorized that a compound(s) volatile in nature was removed and is contributing to wastewater toxicity.

pH Adjustment Test. A wastewater's pH has a substantial effect on the solubility, polarity, volatility, stability, and speciations of a compound. The pH of a wastewater sample is adjusted to pH 3 (acidic), pHi (initial pH of sample), and pH 11 (basic), and then returned to the original pH. These adjustments are made with sodium hydroxide (NaOH) and hydrochloric acid (HCl). A toxicity test is conducted on each of these samples, and results are compared to the toxicity exhibited in the baseline test. The pH adjustment test also serves as a control to other Phase I tests that require pH adjustments before additional sample manipulation.

Filtration Test. Wastewater at pH 3, pHi, and pH 11 is filtered through 1.0-μm glass microfiber filter paper to provide information on effluent toxicants associated with filterable material. Test organisms are exposed to the filtrate, and survival and sublethal responses are documented. In addition, the filtration test information is needed to complement the octodecyl carbon (C_{18}) solid-phase extraction carbon adsorption test, which requires use of filtered wastewater.

Ethylenediamine Tetraacetate Chelation Test. If toxicity is attributable to cationic metals (other than anions such as selenium, arsenic, and chromate), the addition of a chelating agent, ethylenediamine tetraacetate, may render them biologically unavailable. Ethylenediamine tetraacetate is added over a range of concentrations so that a discernible toxicity pattern will be exhibited if cationic metals are responsible for wastewater toxicity.

Oxidant Reduction Test. Sodium thiosulfate, a strong reducing agent, is added to wastewater samples to determine if oxidants such as chlorine, bromine, iodine, ozone, chlorine dioxide, and chloramines are contributing to wastewater toxicity. A dilution series of increasing amounts of sodium thiosulfate is added to effluent aliquots. The appropriate test design includes addition of enough sodium thiosulfate to reduce any oxidants but not enough to cause reagent-induced toxicity. Following addition of sodium thiosulfate, toxicity tests are conducted on these solutions.

Aeration Test. Three wastewater aliquots are adjusted over a pH range of 3 to 11 and are aerated to remove any toxicants through volatilization or oxidation. Samples are adjusted back to the original pH, and toxicity tests are conducted with the manipulated wastewater samples.

Octodecyl Carbon Solid-Phase Extraction Test. Wastewater adjusted to three pH levels (pH 3, pHi, and pH 9) is passed through a prepared octodecyl carbon (C_{18}) filtration column for removal of nonpolar organic compounds or those compounds rendered nonpolar through pH adjustment. Nonpolar compounds are retained on the columns, and the aqueous passthrough eluates are used in toxicity tests.

Graduated pH Test. The graduated pH test was originally developed to address ammonia toxicity; however, it also characterizes other chemicals whose toxicity is pH dependent (for example, metals or cyanide). Ammonia, especially in municipal treatment plants, is prevalent and is toxic at relatively low concentrations. The toxicity of ammonia is pH and temperature dependent, with two basic forms of ammonia, ionized (NH_4^+) and un-ionized (NH_3).

Although the un-ionized form is typically more toxic, there are conflicting effects of pH on ammonia toxicity. While increasing pH increases the amount of the un-ionized form, a dilution factor must be calculated to account for the increased toxicity resulting from the increased concentration of un-ionized ammonia and the decreased toxicity of un-ionized ammonia resulting from an elevated pH. Similar factoring must be considered when a decrease in pH reduces the amount of the un-ionized form but increases the toxicity of un-ionized ammonia because of a lesser pH (U.S. EPA, 1993a). Three effluent aliquots are adjusted to pHs of 6, 7, and 8, and a toxicity test is conducted. Based on the chemical equilibrium of ammonia, higher toxicity should be associated with the effluent sample adjusted to a pH of 6 if ammonia is the cause of toxicity.

In general, all of the tests in the Phase I series are conducted concurrently. This allows for valid comparisons of the tests to the one baseline test. If additional sample manipulations are conducted and tested on a different day, another baseline test with unmanipulated whole effluent is set up in parallel

for comparison and to determine if the magnitude of toxicity changes with storage time (at 4°C in the dark).

If, for example, a TIE is conducted, and comparisons of all tests, except the C_{18} solid-phase test, are similar to the baseline value, then one can conclude that constituents such as metals, volatile compounds, oxidants, and ammonia were probably not responsible for wastewater toxicity. If toxicity decreases compared to the baseline following treatment with C_{18}, the responsible toxin is most probably one nonpolar organic compound or more. An example of such a data set is shown in Table 6.2. The C_{18} column can then be sequentially eluted with aliquots of increasing concentrations of methanol (for example, 50, 75, 80, 85, 90, 95, and 100%) to elute different logP nonpolar organic compounds in separate fractions for toxicity testing so that the range of suspect toxicants is narrowed. This scenario exemplifies the strategy of a TIE test, which narrows the possibilities until the actual toxicant is identified.

Table 6.2 Sample Phase I effluent characterization results

Test/fraction	48-hour LC_{50}
Baseline	43.8
Filtration	44.0
Aeration	40.1
C_{18} SPE[a]	>100.0
Oxidant reduction	43.8
EDTA[b] chelation	43.8

[a] SPE = Solid phase extraction.
[b] EDTA = Ethylenediamine tetraacetate.

PHASE II—TOXICANT IDENTIFICATION PROCEDURES. Once effluent variability has been established and consistent results are obtained over a number of Phase I tests, Phase II toxicant identification procedures may be initiated. Several specific compounds are targeted by Phase II procedures. U.S. EPA has published methods for identification of nonpolar organics, metals, and ammonia (U.S. EPA, 1993a). During Phase II toxicant identification, the toxic fractions of the group of compounds identified during Phase I (that is, nonpolar organics) are separated, concentrated, and identified. Analytical instrumentation such as gas chromatography/mass spectrophotometry (GC/MS), high-pressure liquid chromatography, and atomic adsorption may be included in Phase II evaluations. However, aquatic toxicity testing remains the key measure.

The major objective of Phase II is to fraction the toxic portion of the wastewater from the nontoxic portion, conduct toxicity tests, evaluate the results, and move a step further in the toxicant identification process. There is a tendency, however, to use expensive GC/MS analysis prematurely in the Phase II

process. Effluent is a complex waste, and if nontoxic constituents are not satisfactorily separated from the toxic portion, a chromatograph will be difficult to interpret because nontoxic peaks will mask the true toxic constituents.

For a more detailed description of Phase II toxicant identification procedures, refer to U.S. EPA's *Methods for Aquatic Toxicity Identification Evaluations: Phase II: Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity* (1993a), which describes identification procedures of nonpolar organics, metals, and ammonia.

In any TIE test design, adequate controls and blanks to detect artifactual toxicity are needed. Also, broad assumptions should be avoided where possible. For example, zeolite, a natural resin that is effective in ammonia removal, is used during Phase II testing to implicate ammonia as the toxicity causative agent. If toxicity of a wastewater is eliminated following zeolite treatment, it may appear that ammonia is the cause of toxicity. However, zeolite is not specific to ammonia and may remove compounds other than ammonia.

PHASE III—TOXICANT CONFIRMATION PROCEDURES. Phase III, or confirmation procedures, is used to verify that the identified toxicant is the true cause of toxicity. Typically, Phase III will follow work completed in Phases I and II. The final confirmation not only serves to prove that the suspected compound(s) is in fact the toxicity causative agent(s), but also increases the confidence of success when a toxicity control option is selected.

Phase III approaches such as correlation, symptom, species sensitivity, spiking, and mass balance are described in detail in U.S. EPA's *Methods for Aquatic Toxicity Identification Evaluations: Phase III: Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity* (1993b).

The importance of confirming the true causative agent(s) of toxicity is manifested when extensive treatment plant modifications such as carbon adsorption are under consideration. Identification of the actual toxicant(s) is desirable because remediation may involve something as simple as product substitution or minor treatment plant efficiency upgrades. However, identification of the toxicants may be impossible in some cases because of the complexity of the wastewater. Therefore, treatability procedures based on a whole-effluent approach may be considered. Typically, remediation from this standpoint would be expensive.

Some laboratories may lack the diversity of testing skills needed to identify the correct compound, and considerable sums of money have been expended on failed identification efforts. It is a good idea for facilities to develop a TIE plan that includes what TIE laboratory they would use if needed and investigate the laboratory before requiring its services. When a toxicity event occurs, time is often an important factor in determining whether the TIE is successful and in finding its source.

Because the cost of a TIE can vary from hundreds to thousands of dollars and multiply quickly, some critical issues should be considered before initiating a TIE. Some of the issues are discussed below.

Effluent Variability. Because effluent can vary in chemical composition over a period of time, particularly in the case of WWTPs, results from one set of TIEs performed on samples collected at one point in time may not provide sufficient information about the facility's effluent toxicity problem. Further, it is possible that different toxicants are responsible for the toxicity or aggravate the toxicity of the final effluent at different times of the year. For example, a company responsible for pharmaceutical intermediates may produce different byproducts daily. Possibly, toxicity associated with only monthly housekeeping procedures are the cause of wastewater toxicity. Toxicity may also vary because of treatment plant efficiency, and treatment plant efficiency may vary with the time of day or season.

The issue of effluent variability can be particularly challenging for WWTPs, and in most cases, the effluent variability is a function of the size of the facility. Assessing the source of variability is likely to be more challenging for larger metropolitan WWTPs with numerous industrial users because it may be necessary to evaluate each significant flow contributor or at least those that are suspected of contributing problematic compounds from time to time. If variability is not properly addressed, one runs the risk of designing a reduction solution that misses the target and fails to bring the permittee into compliance during the periodic toxic flows.

Many factors may contribute to wastewater variability, such as differing product manufacturing processes. Therefore, more than one complete Phase I toxicant characterization series is recommended. The exact number of tests to be conducted is facility-specific. Effluent collection times such as 24- and 8-hour composites should be varied for each Phase I test; varying the day of the week and the season of the year is also advisable. Consistent characterization results of a number of effluent samples are needed before Phase II toxicant identification procedures are initiated.

Whole Effluent Versus Individual Waste Streams. One of the most critical issues is whether to perform the TIEs on samples of final effluent or individual contributing waste streams. It is recommended that preliminary toxicity screening be conducted on individual waste streams and toxicity test results be used to rank the waste streams. Toxicity identification evaluation manipulations can then be considered on the most toxic waste streams only. This will reduce costs and help focus the toxicant identification effort on the areas most likely to respond to pretreatment to reduce the overall final toxicity level.

It should be mentioned that data from collection systems samples may be misleading because constituents are in a different concentration than they would be when diluted and found in effluent. An example of this would be

during a test of a collection system when a particular branch may appear to be the one causing the toxicity because its contents contain a toxicant in borderline toxic concentration. When diluted at the treatment plant, its concentration is no longer toxic. On the other hand, the branch that is actually contributing the toxicity may not appear to be the cause because its contents need to react with conditions at the treatment facility or the receiving water to become toxic. Additionally, collection system or influent toxicity has a high microorganism load that can also cause false-positive indications of toxicity.

TOXICITY SOURCE EVALUATION

If TIEs are conducted on samples of the final effluent only, evaluating the sources of toxicity becomes the next logical step. Results of the TIE will be used to identify possible sources that may be contributing the toxicant (or groups of toxicants) to the wastewater. This task is best accomplished by a team comprising members from each facet of the facility's operations. Each source must then be individually evaluated, no matter how remote it seems to be in relation to the final effluent.

Proper characterization of process or contributory streams is critical in evaluating sources of toxicity. Each stream should undergo chemical-specific analyses and/or direct toxicity measurement. Chemical-specific analyses can be used effectively only when the compounds responsible for toxicity within the final discharge have been clearly identified. If specific identification is not possible or is unclear, a toxicity-based approach is used for each contributing waste stream. Toxicity screening does not have to include full 96-hour acute or 7-day chronic tests. Abbreviated testing often suffices in evaluating potential toxicity sources.

In addition to straightforward analyses of process streams is the need to evaluate raw material use, process byproducts, plant operations, and sanitation practices. A number of cases can be cited in which industrial-grade floor cleaners were the principal cause of final effluent toxicity. In other cases, biocides used within air conditioning systems have found their way into wastewater streams. Most importantly, all potential sources must be evaluated.

TOXICITY CONTROL SELECTION

Once variability has been addressed, and the toxicant(s) has been identified or the source or cause has been determined, the next step is to identify the most cost-effective control option. Again, identification of the actual toxicant(s) may not always be feasible from a cost or technology standpoint. In such cases, proper characterization to determine the source or cause may be sufficient to select a suitable toxicity control option.

Depending on the nature of the toxicant(s) and the source, there are two control options that can be evaluated. The first option, source reduction, is becoming increasingly attractive given waste minimization/pollution prevention incentives and the option's comparatively lower cost. Source reduction involves one or more of the following: substitution of raw materials, improved housekeeping, resource recovery, and alterations in production processes.

Source reduction is most attractive when the actual source of toxicity is confirmed. This does not necessarily mean the actual toxicant is identified, but confirmation of its source(s) is vital. Once the source has been confirmed, process modifications, pretreatment, or some other preventive measure can be evaluated. If the actual toxicant(s) can be identified, product substitution can be assessed, as well as isolation and targeted "upstream" treatment.

Treatment just before final discharge (that is, end of pipe) is the second option in rectifying wastewater toxicity. If the nature of the toxicant or the source is such that waste minimization is not a feasible option, then additional or modified treatment is the only avenue to reduce whole effluent toxicity to acceptable levels.

Certainly, control options differ for industrial versus municipal permittees. Municipal WWTPs must consider multiple influent sources and evaluate the effectiveness of upstream pretreatment programs versus upgrades or improvements to the municipal treatment system itself. Pretreatment control is typically only feasible when sufficient source data exist. These data should provide adequate information on the variability of the industrial users' wastewater characteristics, their contribution to the overall toxicity problem, and their plans for future process or product changes. In-plant control options must be examined when insufficient data exist on influent or other reasons dictate the need for direct final effluent toxicity control. These in-plant control options may include treatment modifications such as dechlorination, biological treatment optimization, or advanced wastewater treatment systems such as carbon or resin adsorption.

TOXICITY CONTROL IMPLEMENTATION

Once the appropriate control option is selected, the feasibility of implementation is considered. Issues like cost of operating the recommended control, ease of installation and operation, time frame, and disruption in normal facility operation will have to be evaluated before actual implementation. Whatever control method is selected, confirmation of its ability to bring the permittee into compliance for toxicity must be *confirmed* before full-scale implementation. Bench-scale and, if necessary, pilot-scale demonstration of the control method in a repetitious manner designed to address temporal variability can save costly pursuit of an ineffective control measure.

Conclusion

This chapter provided a brief overview on decisions related to conducting toxicity reduction evaluations. An important fact to keep in mind is that no two TRE studies are alike. Conditions of the treatment processes, wastewater components, receiving water streams, discharge toxicity standards, flow rates, and a number of other factors vary significantly from site to site. Given the severe implications of noncompliance, the multiple options and costs associated with toxicity control measures, and the complexities of identifying the source of the problem, most permit holders solicit assistance from outside consultants and wastewater engineering firms. Assistance from experienced toxicity reduction specialists can be invaluable for a timely, therefore cost-effective, resolution to a wastewater toxicity problem.

In selecting a contractor or consultant for this type of work there are some important considerations that should be weighed with the overall cost for the service. There are a number of firms nationwide that are capable of supporting a TRE study by providing toxicity monitoring, engineering design, process engineering, or other disciplines critical to a full study. Ideally, a firm should be selected that can offer a full multidisciplinary approach. These firms are staffed with teams of specialists with expertise in toxicology, process engineering, treatment technologies, inorganic and organic chemistry, engineering design, wastewater fate and effects modeling, permit negotiations, cost/benefit analysis, and other appropriate disciplines.

Finally, although outside consultants can be invaluable, the more knowledge that a private discharger or WWTP has about the facility's process streams and wastewater treatment history, the easier it will be to implement a corrective action. No outside expert is more valuable than a knowledgeable in-house plant engineer or operator. Given their historical knowledge of influent and effluent characteristics and possible industrial process charges, in-house hands-on personnel can significantly reduce the time and effort required to implement a wastewater toxicity corrective action. A savings of time and effort can convert to a savings in cost.

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Chapter 7
Case Studies

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CASE STUDY 1—MUNICIPAL DISCHARGES

A wastewater treatment plant (WWTP) in the midwestern U.S. discharging approximately 7.5 ML/d (2 mgd) treats influent consisting of approximately 10% industrial wastewater and 90% residential wastewater. Final effluent discharge is to the headwaters of a small stream, where the WWTP effluent makes up the entire flow during dry periods. The WWTP personnel are responsible for the plant's industrial pretreatment program, which has seven significant industrial users (SIUs). No significant violations of categorical standards for SIUs have occurred since the pretreatment program was

initiated. Loadings of priority organic pollutants are low and are seldom detected in either the plant's effluent or its biosolids.

Despite its exemplary pretreatment program, the WWTP often exceeded its National Pollutant Discharge Elimination System (NPDES) permit limits for copper and mercury, both of which were at or near analytical detection levels. A pollutant loading study showed that most of the influent copper originated from residential sources, while mercury appeared to originate primarily from industrial discharges. The WWTP initiated a plan to reduce influent loading of mercury from industry. However, even with all sources controlled to the maximum possible extent, violations of copper and mercury limits were still likely (Indiana Department, 1989).

Copper limits in the WWTP's permit were based on potential toxicity to freshwater organisms, while mercury limits were based on potential to bioaccumulate in fish to levels unsafe for human consumption. The WWTP personnel commissioned a biomonitoring study to determine whether different limits could be justified that protected receiving stream uses but were easily achieved. The following biomonitoring techniques were used in the study:

- Whole-effluent toxicity tests,
- Effluent spiking with copper and testing with sensitive freshwater animals to determine copper toxicity thresholds,
- Fish tissue analysis to determine whether mercury is bioaccumulating in resident species, and
- Biological surveys of fish and macroinvertebrates to determine whether pollution-sensitive forms are affected by the discharge.

Quality assurance elements in the studies included

- Conducting toxicity tests according to good laboratory practice requirements,
- Maintaining chain-of-custody records for all samples,
- Using monthly reference toxicant tests to ensure the sensitivity of the test animals, and
- Using duplicate samples in biological surveys to ensure that reproducible results were being obtained.

RESULTS OF COPPER STUDIES. Chronic toxicity of the effluent was estimated using the U.S. Environmental Protection Agency's (U.S. EPA's) short-term chronic tests with *Ceriodaphnia dubia* and fathead minnow larvae. Both species are known to be adversely affected by copper in chronic laboratory tests when concentrations exceed approximately 20 µg/L in hard water. Three separate tests with both species showed that undiluted effluent was not chronically toxic to either test animal even though copper exceeded NPDES limits in each test. These results provided the first biological evidence that the WWTP's copper limits were overly protective.

Additional toxicity tests were conducted by spiking effluent with reagent-grade copper sulfate to determine a toxicity threshold. A chemically simple synthetic water with identical hardness was also spiked with copper. Two different test animals (*Daphnia magna* and fathead minnows) were used to determine copper effects in each water type. The results showed that at least twice as much copper was required in effluent as in synthetic water to cause the same amount of lethality. These results provided additional evidence that original copper limits were overly protective.

A biological survey of the WWTP receiving stream showed that the stream was dominated by animals that are tolerant to low dissolved oxygen levels (fish such as creek chubs and green sunfish and benthic invertebrates such as sludge worms, snails, and isopods). Similar conditions were observed both upstream and downstream from the WWTP outfall. The dominance of wastewater-tolerant forms was probably caused by raw wastewater overflows from a leaky interceptor upstream from the plant. Nevertheless, many of the common species present in the stream are known from the scientific literature to be sensitive to copper (for example, creek chubs and the snail *Physa* were both abundant but exhibit acute mortality at 43 to 84 µg/L copper, which is only slightly higher than the WWTP's chronic-based limit). Therefore, copper in the WWTP effluent appeared to be having little effect on these copper-sensitive species. This provided more evidence that copper limits were probably unnecessarily strict to adequately protect aquatic life in the stream.

The preponderance of biological evidence demonstrated that the WWTP's copper limits could be relaxed somewhat without impairing aquatic life in the stream. The biomonitoring results were used to justify an adjustment of the WWTP's monthly average copper limit upward by a factor of 2.

RESULTS OF MERCURY STUDIES. Because the limit for mercury is based on mercury's potential to bioaccumulate, fish tissue samples from three different species (green sunfish, yellow bullheads, and creek chubs) were collected from the receiving stream immediately below the WWTP discharge on four separate occasions over 3 years and analyzed for total mercury concentrations.

The results showed that mercury concentrations in both whole-fish and fillet samples ranged between 0.047 and 0.13 mg/kg. There was little difference between older and younger fish of the same species. The observed values were well below the U.S. Food and Drug Administration's (U.S. FDA's) action level for mercury and compared favorably with levels from fish collected at background sites with no known point sources of mercury inputs.

Because new biological evidence demonstrated that mercury was not accumulating in fish tissue, the WWTP's mercury limit was also shown to be unnecessarily stringent. The WWTP was allowed to drop its monitoring program for mercury in water but continues to monitor twice yearly for mercury in fish tissue.

CASE STUDY 2—INDUSTRIAL DISCHARGES

The Grand Calumet River Basin in northwestern Indiana contains one of the world's largest concentrations of heavy industry. Originally created as a drainage project, the Grand Calumet River is now approximately 25 km long and flows via the Indiana Harbor Canal to southern Lake Michigan. At least 90% of the river's flow consists of treated industrial wastewater. The remaining portion of the river's flow is composed of municipal wastewater, stormwater runoff, and combined sewer overflows.

During the past 100 years of human activity in the watershed, the Grand Calumet's water, sediments, and biota have become polluted. Chemical monitoring conducted since the 1950s has shown high levels of metals, cyanide, ammonia, phenolics, polycyclic aromatic hydrocarbons (PAHs), volatile organic chemicals, and polychlorinated biphenyls (PCBs) in water and/or sediment and biota of the river. Because of the magnitude of contamination, the Grand Calumet River has been classified as a Class A area of concern (highest priority for cleanup) by the International Joint Commission, which regulates water issues in the Great Lakes area of the U.S. and Canada.

Biomonitoring has played an important role in the decision-making process of this cleanup activity. Biomonitoring studies have included whole-effluent toxicity tests, sediment toxicity tests, toxicity identification evaluations, monitoring of fish and macroinvertebrate communities, and fish tissue residue studies. As detailed below, biological techniques have been used to identify where continuing toxicant loading is occurring, which toxicants are causing the greatest problems, and ways that remediation might be accomplished most effectively (Simon, 1988).

Biological studies in the Grand Calumet River Basin, conducted by numerous investigators over 20 years or more, have incorporated a wide variety of quality assurance techniques. Only within the past 5 years or so have quality assurance procedures been well documented. For example, studies funded by U.S. EPA have recently required a quality assurance project plan (QAPP) to be submitted before funding. The QAPP typically consists of a written document detailing the following:

- Standard operating procedures to be used,
- Sampling and analysis by approved methodology,
- Documentation that instruments are properly calibrated,
- Maintenance of chain-of-custody records,
- Assurance that representative samples are collected,
- Determination of necessary sampling precision and data quality objectives,
- Use of adequate sample replicates,

- Accurate and timely recordkeeping, and
- Assurance that participating personnel are qualified to do the required work.

RESULTS OF WHOLE-EFFLUENT TOXICITY TESTS. Recent whole-effluent toxicity tests of 19 point source discharges in the watershed showed that no discharges were acutely toxic. However, approximately half of the discharges tested have been chronically toxic to either fathead minnows or *Ceriodaphnia*. Suspected toxicants included cyanide, ammonia, total dissolved solids, and perhaps metals. Because most of the river's flow consisted of wastewater, dilution flow was negligible, and continued efforts to further reduce point source toxicity were still necessary for some discharges.

Toxicity tests of combined sewer overflows and stormwater runoff in the watershed showed that nonpoint sources were still contributing to toxicant loading. For example, more than half of the combined sewer overflow discharges and 15 to 20% of monitored stormwater sites were acutely toxic to one or more species. Concurrent chemical sampling indicated that metals and cyanide were probably not responsible for observed toxicity, but ammonia loading from these sources appeared to be significant.

RESULTS OF SEDIMENT TOXICITY TESTS. Sediment toxicity tests have shown that all tested sediments in the basin were toxic to one or more test organisms. However, some sites were more toxic than others. A sediment toxicity identification evaluation showed that ammonia was probably the most widespread and lethal sediment toxicant, but PAHs (phenanthrene and naphthalene) were important toxicants downstream from a large steel mill.

Another approach to sediment toxicity testing showed that exposure of Grand Calumet River sediment to ultraviolet light increased its toxicity. This observation is consistent with PAH toxicity because several PAH compounds are known to have their toxicity activated by ultraviolet light. The study was also important in making decisions about sediment remediation. For example, dredging may expose suspended sediments to sunlight, activating the PAHs and increasing instream toxicity.

A third approach to sediment testing showed that whole sediments from the basin were more toxic to test organisms than elutriates. This suggests that some important toxicants remain tightly bound to sediments.

RESULTS OF BIOASSESSMENTS. Fishery studies have demonstrated that water quality in the basin has improved significantly during the last decade. Incapable of supporting fish during the early 1980s, the Grand Calumet River now has a resident community of up to six to eight fish species, with a total of 21 species collected at one time or another within the basin. Most of this improvement is thought to be a result of upgraded municipal wastewater treatment facilities in the watershed. Levels of dissolved oxygen

present in the stream have increased significantly following the decrease in organic loading.

Despite recent improvements in the number and diversity of species present, however, the fish community, as measured by the index of biological integrity, has remained poor. Abnormalities (eroded fins, lesions, external tumors) have commonly been observed in fish from the river. Continued fishery studies are planned as a way of documenting further water quality changes in the basin.

Macroinvertebrates, which are less mobile than fish and more representative of local conditions, have also been collected regularly within the basin over a period of 15 years. The macroinvertebrate studies have mirrored results of fishery studies, showing improvements in water quality over time. However, impairment of the macroinvertebrate community from both organic pollution and toxic substances is still evident at all sites monitored. These studies have also provided evidence that metals and cyanide are probably not responsible for most of the biological impairment. Instead, PAHs associated with steel-making activities may be more important toxicants. New NPDES permits for several dischargers in the basin now contain numerical limits for several PAH compounds to reduce PAH loading.

RESULTS OF TISSUE RESIDUE STUDIES. Tissue residue studies have shown that some contaminants are significantly accumulating in resident biota. The PCB and chlordane concentrations in whole fish samples typically exceed U.S. FDA action levels, which are used to trigger fish consumption advisories. Twenty-seven other organic contaminants, which are seldom detected elsewhere in healthy aquatic systems, have also been detected in one or more fish samples from the Grand Calumet River Basin. Crayfish and aquatic macrophytes, which are more representative of local conditions, are also accumulating some of these contaminants. The results have been used to prioritize sites for cleanup.

SUMMARY. Numerous industrial discharges in the Grand Calumet River Basin have caused water quality problems. Biomonitoring techniques have been used to identify the most significant toxicants, determine sites where continuing toxicant loading exists, prioritize sites for cleanup, and document improvements in the watershed. In addition, biomonitoring has been important in making decisions about the most effective remediation techniques to be used in the cleanup process.

Case Study 3—Watersheds

The Stillwater Wildlife Management Area in southwestern Nevada was established in 1948 to provide aquatic habitat for fish and waterfowl. The preserve receives water from rainfall, irrigation drain waters, and groundwater. Aquatic

habitat in the area has been reduced by more than 70% in 80 years because the quantity and quality of water reaching the wetland areas have declined because of increased irrigation. Sport fisheries present in the 1950s and 1960s have collapsed, and many bird and fish kills have been documented recently.

Concerns about the declining water quality of the area prompted a study to help initiate corrective actions (Dwyer *et al.*, 1992). Biomonitoring techniques were used to determine whether irrigation water draining into the preserve contained toxic substances that are potentially detrimental to the habitat of the area. A second goal of the program was to distinguish effects caused by salinity in irrigation water from those caused by other toxicants. For quality assurance purposes, the study included use of 10% blanks and 20% blind replicates and spike samples for all chemical analyses. Toxicity tests were conducted according to standardized procedure, and no test data were used in which control mortality exceeded 10%.

Researchers sampled water from four sites in the management area and tested each sample for acute toxicity to four aquatic species: *Hyalella azteca* (amphipod) and striped bass, which are tolerant to high salinity, and *Daphnia magna* (crustacean) and fathead minnows, which are intolerant to high salinity. Water from two sites was not toxic to any species. Water from one site was toxic to the two salinity-intolerant species but not to the two that tolerate high salinity. Water from the fourth site was toxic to all four species. Concurrent chemical sampling confirmed that the site toxic to salinity-intolerant species had high chloride and sulfate concentrations. Therefore, contamination from other unknown chemicals was unlikely there.

However, one site toxic to all animals had water chemistry similar to the sites where salinity-tolerant animals survived. Therefore, the presence of another toxicant or environmental factor was thought to be present at this location. To determine whether certain trace elements present in the water (lithium, arsenic, boron, molybdenum, and strontium) contributed to the observed toxicity at this site, researchers used a synthetic water with a major ionic composition similar to that observed in the field but without any additional trace elements. They exposed the salinity-tolerant species to this water and an identical reconstituted water with trace elements added. Researchers reported 96-hour lethal concentration 50% (LC_{50}) values for striped bass of 70% in synthetic water without trace elements added and 20% in synthetic water with trace elements added. For the amphipod, the results were 96-hour LC_{50} values of 60% in the synthetic water without trace elements and 30% in synthetic water with trace elements added. These results showed that trace elements increased to toxicity of the water, but that the water without trace elements was still toxic even though salinities were within the range of tolerance to these test animals. Researchers suspected that at least some of the adverse effects on these aquatic species were caused by atypical ionic ratios causing osmotic imbalance in the animals rather than by ordinary chemical toxicity.

This was confirmed when the researchers added calcium to the original synthetic water mixture. With each increase in calcium concentration, the water became less toxic to striped bass. The 96-hour LC_{50} values for striped bass were reported as follows: 70% at low hardness, 85% at medium hardness, and greater than 100% at high hardness. Once the ratio of calcium to other major ions reached a certain level, the water was suitable for striped bass survival. However, when trace elements were added back to the water, toxicity increased again. The researchers concluded that both trace elements and atypical ionic composition of the water were causing at least one site in the Stillwater Wildlife Management Area to be detrimental to aquatic life.

The biomonitoring information obtained in this study could be useful in the future management of the area. For example, the biomonitoring information could be used to prioritize areas for attention. Watersheds feeding the two nontoxic sites did not appear to need immediate attention. In contrast, the watershed draining into the site toxic only to salinity-intolerant animals needed management of chloride and sulfate sources. The fourth site, toxic to all species, might be improved by controlling both salinity and trace element sources.

CASE STUDY 4—CHEMICALS IN THE MARINE ENVIRONMENT

Oil spills are a byproduct of the modern age, occurring frequently in the marine environment as oil is shipped by large tankers from production fields to refineries to consumer markets. To help ameliorate the detrimental effects of oil on exposed animals, chemical dispersants are often used to break up oil into less harmful forms. However, there is concern that the dispersants themselves may have harmful environmental effects. In fact, the earliest dispersants were often more toxic to marine life than the oil they were intended to treat.

Biomonitoring techniques were needed to help those responsible for oil spill cleanups and various regulatory agencies to decide which types of dispersants were both effective in treating oil and relatively nontoxic to marine organisms. Aquatic toxicity testing has been conducted on these chemicals in the past 10 years, and the results have shown that toxic concentrations vary from one dispersant to another. A biomonitoring study by researchers in California provided the basis for decision making about which types of oil dispersants were most effective in the marine environment likely to be affected by oil spills along the California coast (Singer *et al.*, 1994).

Researchers used four different test organisms representing a wide range of taxa and ecological niches: a fish common in California bays and estuaries (the topsmelt, *Atherinops affinis*), an edible mollusk (the red abalone, *Haliotis rufescens*), a shrimp (*Holmesimysis costata*), and a common aquatic plant along the California coast (the giant kelp, *Macrocystis pyrifera*). By testing a

diversity of organisms, researchers hoped to provide information that would protect a broad range of marine life from the toxic effects of various oil dispersants. Quality assurance elements of the testing program included use of three replicates per test concentration, randomization of test organisms within the treatments, hourly chemical monitoring to ensure that test conditions remained similar throughout the test, discarding of results from tests in which control mortality was unacceptably high, and testing of each dispersant three times for each organism to ensure that results remained within acceptable limits of variability.

Two licensed, commercially available dispersants were tested for toxicity. All tests were conducted using acute (48- or 96-hour) exposures because the normal exposure of marine life to dispersants is typically of short duration. The inhibitory concentration 50% (IC_{50}) (for the giant kelp and the red abalone) or LC_{50} (for fish and shrimp) was calculated. A summary of the results is shown in Table 7.1.

Table 7.1 Lethal (LC_{50}) or inhibition (IC_{50}) concentration observed for four species exposed to two licensed dispersants

	Dispersant A, ppm	Dispersant B, ppm
Fish *(Atherinops affinis)* LC_{50}	44–46	48–73
Mollusk *(Haliotis rufescens)* IC_{50}	15–25	21–23
Shrimp *(Holmesimysis costata)* LC_{50}	25–35	118–122
Kelp *(Macrocystis pyrifera)* IC_{50}	73–96	73–79

Results showed that the red abalone mollusk was more sensitive to the two dispersants than other test animals. Tests also showed that dispersant B was somewhat less toxic than dispersant A to all four test organisms, especially to shrimp.

Based on these results, researchers recommended the following:

- Red abalone is the preferred test species on which to base judgments about toxicity effects because it is the most sensitive to the two dispersants; and
- If its oil-dispersing ability is equal, dispersant B is preferable to dispersant A because of its lower toxicity.

CASE STUDY 5—USING LONG-TERM BIOLOGICAL MONITORING DATA TO EVALUATE THE RECOVERY OF DISTURBED SYSTEMS: THE BIOLOGICAL MONITORING AND ABATEMENT PROGRAM, OAK RIDGE NATIONAL LABORATORY

The Environmental Sciences Division Biomonitoring Group at Oak Ridge National Laboratory (ORNL) conducts studies to evaluate the ecological health of aquatic and terrestrial ecosystems near U.S. Department of Energy (U.S. DOE) facilities in Oak Ridge, Tennessee; Paducah, Kentucky; and Portsmouth, Ohio. These biological monitoring programs provide a basis for assessing compliance with environmental regulations, identifying causes of adverse ecological impacts, and evaluating the effectiveness of remedial actions by documenting ecological change. Studies conducted by section staff support a concept of biological monitoring as a combination of traditional and experimental approaches. Established toxicity-testing protocols and conventional ecological surveys are combined with innovative ecotoxicological and biomarker approaches that can be used to assess regulatory compliance and ensure environmental protection and restoration.

Biological monitoring has been conducted in industrially affected streams near U.S. DOE facilities in Tennessee and Kentucky for almost 10 years. A comprehensive biological monitoring and abatement program (BMAP), which was developed to meet NPDES permit requirements, includes (1) toxicity testing; (2) bioaccumulation in aquatic and terrestrial biota; (3) bioindicators of fish health; and (4) fish, macroinvertebrate, and periphyton community surveys. These studies have been used to characterize spatial trends and demonstrate temporal recovery resulting from remedial actions and pollution abatement activities, identify contaminant sources, and provide data for conducting ecological risk assessments. The program uses multiple lines of evidence to evaluate stream recovery and illustrates the importance of using an integrated approach when determining stream health.

An overview of data collected from BMAP studies on East Fork Poplar Creek (EFPC) since 1985 is presented to illustrate the ability of this type of program to document recovery at several levels of biological organization, including individuals, populations, and communities. Effluent from operations at the Y-12 plant, a former nuclear weapons components facility built in the early 1940s, enters the headwaters of EFPC upstream of Lake Reality. From the outfall of Lake Reality to the confluence with Poplar Creek, the stream is

23.4 km in length. East Fork Poplar Creek receives a complex mixture of effluent and nonpoint discharges from the Y-12 plant. Water and sediment in EFPC downstream of the Y-12 plant contain metals, organic chemicals, and radionuclides discharged over many years of operation. Spatial and temporal trends evaluating the importance of historical and ongoing discharges are illustrated by task in the following figures (Figures 7.1 to 7.9). Attributes of each task are highlighted to demonstrate the flexibility of both the program and its components. The development of an integrated database and geographic information systems technology to evaluate the data is also presented. This presentation is an adaptation of a poster compiled for a technical conference (Loar, 1995); consequently, data are presented with little explanatory text.

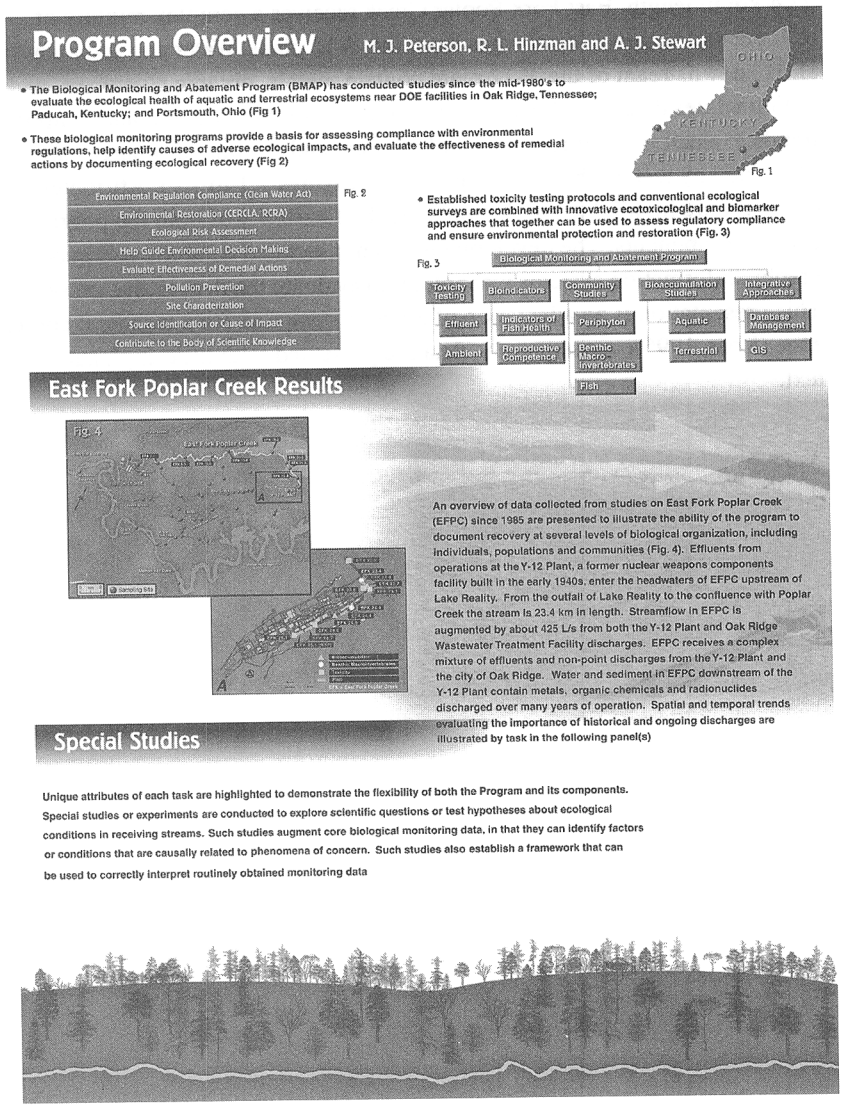


Figure 7.1 Program overview

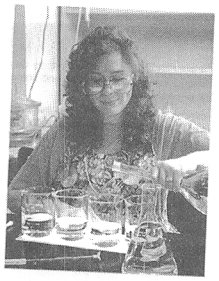
Toxicity Testing

A.J. Stewart, L.A. Kszos, B.K. Beane, P. Braden, M.D. Byars,[1] L.S. Ewald,[2] L.M. Kendrick,[2] K.J. McAfee,[3] G.W. Morris, G.E. Napolitano, M.S. Nazarias,[4] T.L. Phipps,[2] J.E. Richmond, C.D. Runck, W. Session,[2] J.R. Sumner,[3] L.F. Wicker

[1] Midwest Technical Inc., [2] Oak Ridge Research Institute, [3] CKY Environmental, [4] University of Tennessee

Objectives

- Assess long-term changes in pollutant loading to receiving streams by monitoring the toxicity of treated and non-treated wastewaters
- Identify contaminants of concern in toxic wastewaters, thereby guiding operational changes in treatment facilities to improve wastewater quality
- Demonstrate changes in spatial and temporal patterns in biological quality of water that result from environmental remediation actions and improvements in wastewater treatment systems



East Fork Poplar Creek Results

Effluent Toxicity Testing

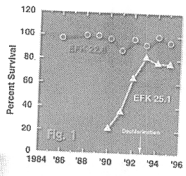
- Two factors accounted for a 17-fold reduction in toxic unit (TU) loading (1986-1988 vs 1988-1992) (Table 1). First, non-treated toxic wastewaters previously discharged to the stream were sent to treatment facilities, where toxicants were removed. Second, new and improved wastewater treatment systems came on line, so that treated wastewaters in general were less toxic than they had been earlier
- Diagnostic acute and chronic toxicity tests identified the following constituents as particular "bad actors" in various wastewaters: uranium; lithium; a fluorescent dye; chlorine; and nickel. In each instance, changes in waste treatment operations were made to reduce releases of the identified constituent

Annual discharge volumes and contributions of toxicity to EFPC by various types of wastewaters from the Oak Ridge Y-12 Plant

Wastewater Source	1986 - 1988 Annual Flow (L/year)	1986 - 1988 Toxic Units[a]	1988 - 1992 Annual Flow (L/year)	1988 - 1992 Toxic Units[a]
Cooling Tower Blowdown	556×10^9	0.12	354×10^9	0.051
Non-treated Wastewaters	113×10^9	3.82	0	0
Treated Wastewaters	138×10^9	0.23	447×10^9	0.187
Total	807×10^9	4.17	801×10^9	0.238

Ambient Toxicity Testing

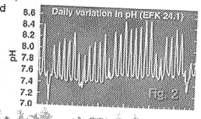
- A longitudinal gradient in ambient toxicity was evident, with sites closer to the Y-12 Plant being more toxic than ambient sites farther downstream (Fig. 1)
- The site farthest upstream (EFK 25.1) has become substantially less toxic through time (Fig. 1). Thus, the ambient testing program has documented improvements in water quality in EFPC, both spatially and temporally





Special Studies

- Special studies are underway to better understand how fine-scale changes in water quality conditions alter toxicant availability
- Hydrosonde[R] instruments are deployed at various sites in the stream to monitor conditions such as temperature, pH, conductivity, dissolved oxygen and turbidity. At some sites, pH varies by more than 1 standard unit over daily cycles due to the photosynthesis of attached algae (Fig. 2). Day-to-night changes in pH of this magnitude are great enough to generate significant day-to-night differences in the toxicity and downstream transport of various metals



- Bomb calorimetry, protein analyses, measurements of the organic matter content in periphyton and drifting particulate matter, and algal pigment analyses by high performance liquid chromatography are used to explore the effects of nutrient enrichment on the ecological energetics of communities in upper EFPC

Conclusions

- A sustained toxicity monitoring program, consisting of effluent testing, ambient water testing, and toxicant identification activities, has demonstrated the value of improvements in waste treatment operations and revealed that water-quality conditions in upper EFPC have improved

Figure 7.2 Toxicity testing

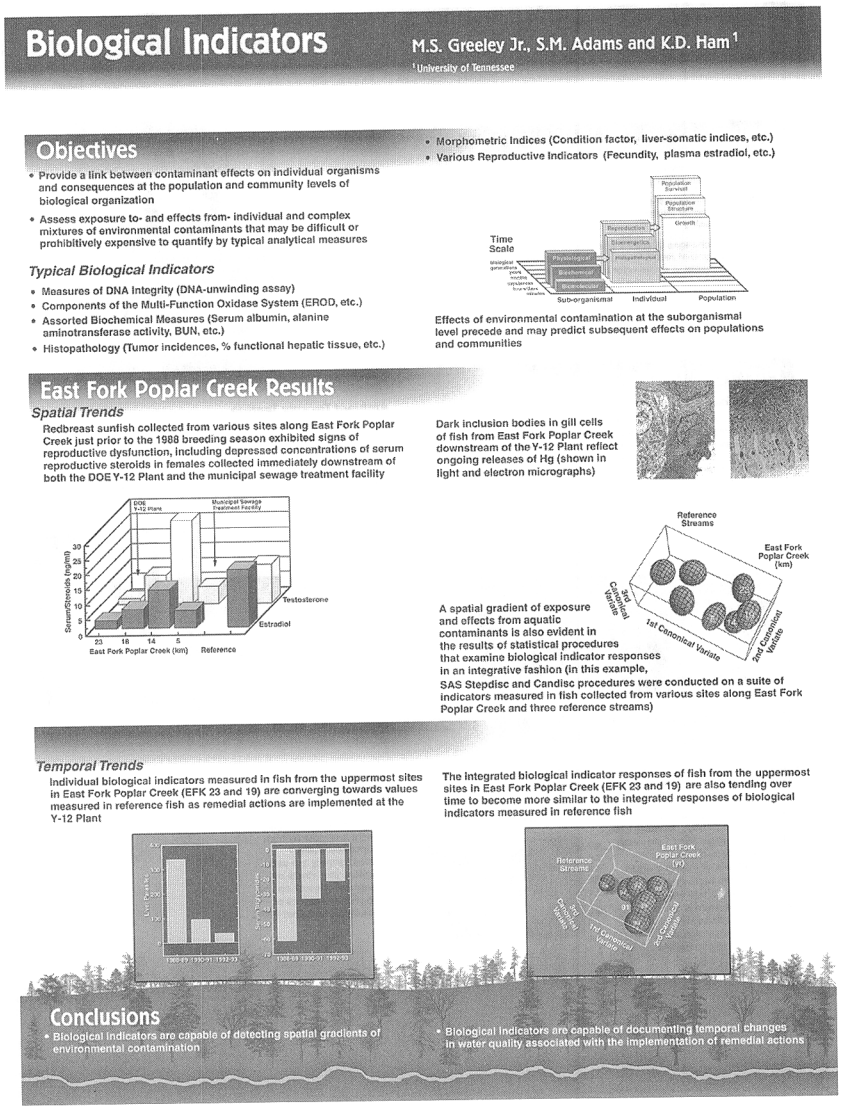


Figure 7.3 Biological indicators

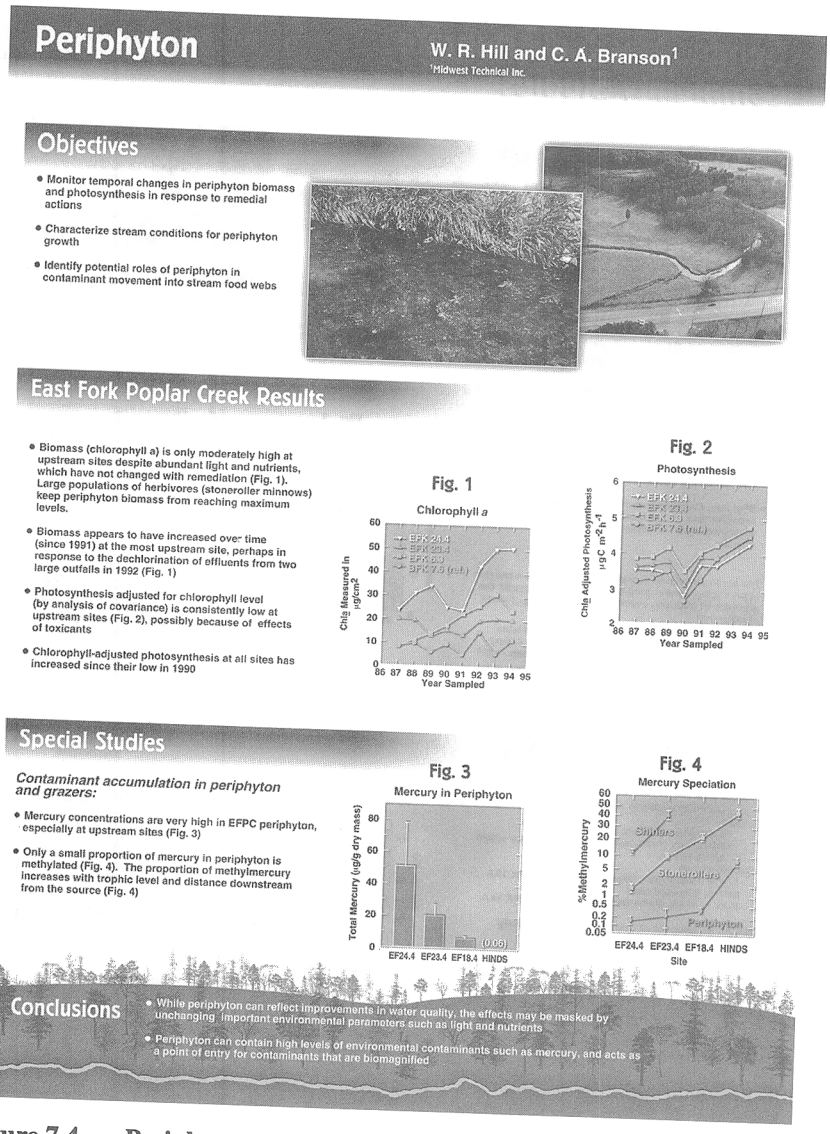


Figure 7.4 Periphyton

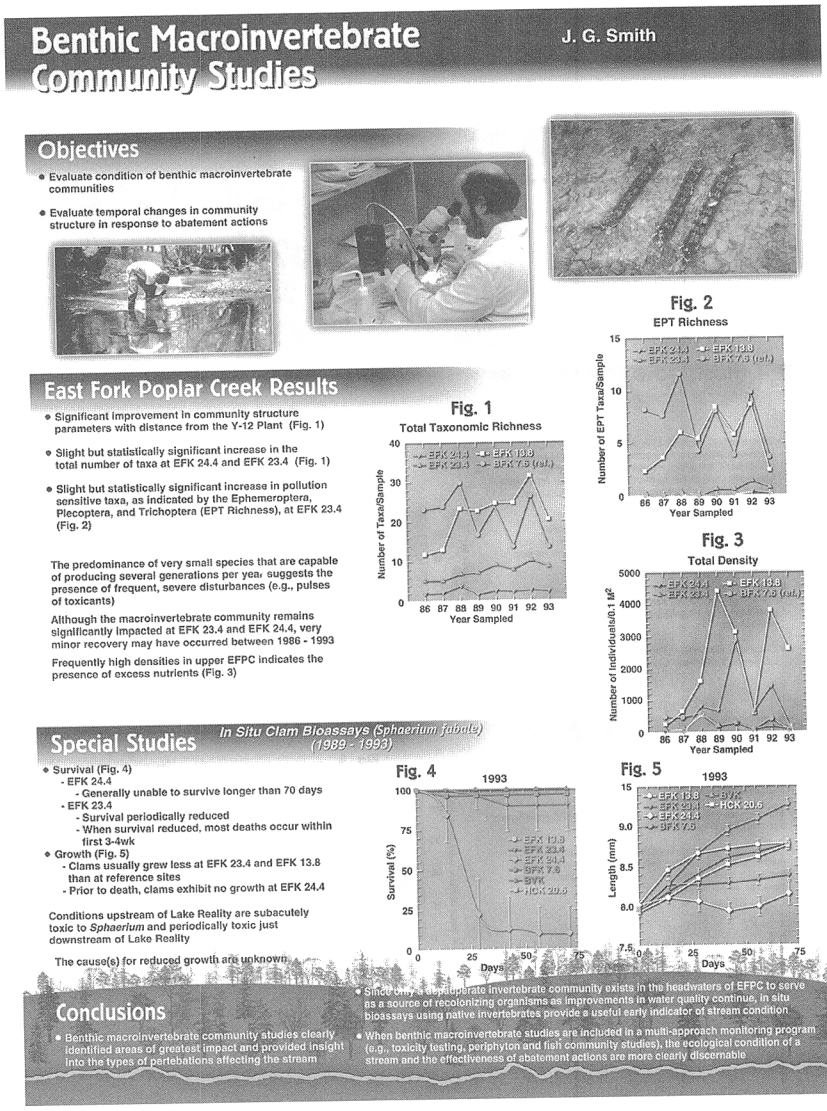


Figure 7.5 Benthic macroinvertebrate community studies

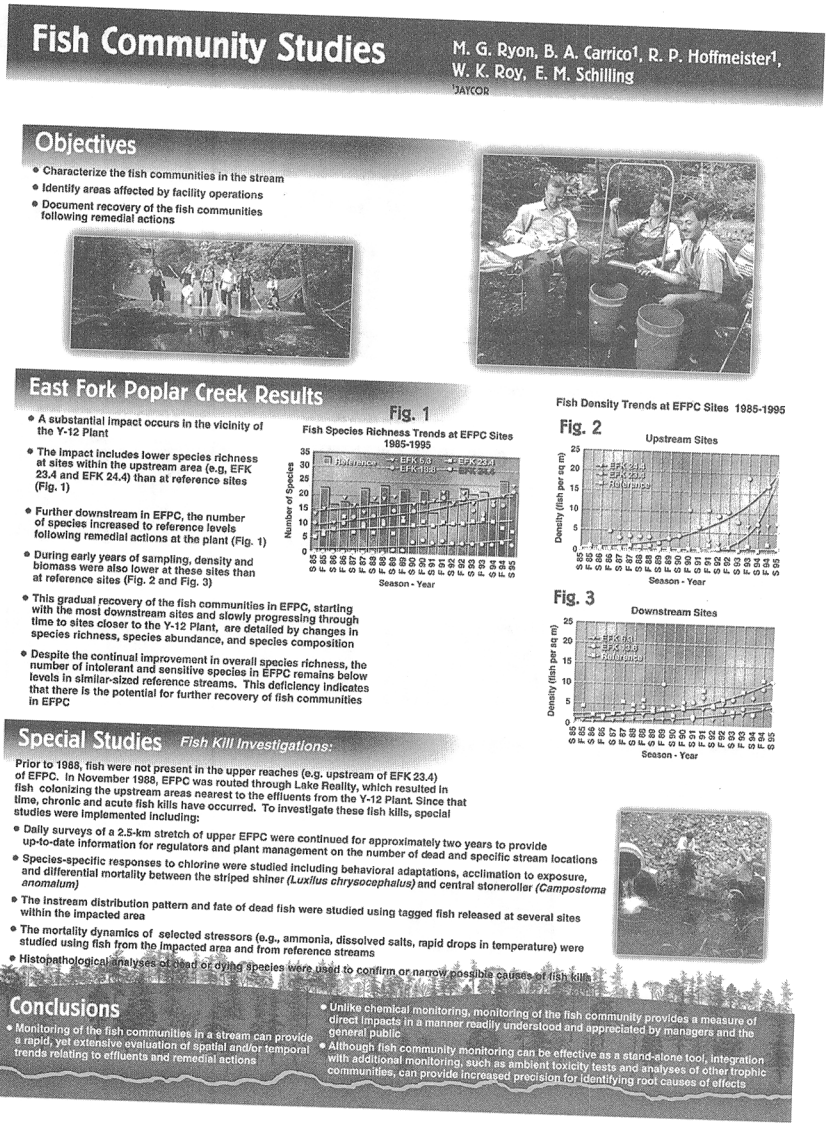


Figure 7.6 Fish community studies

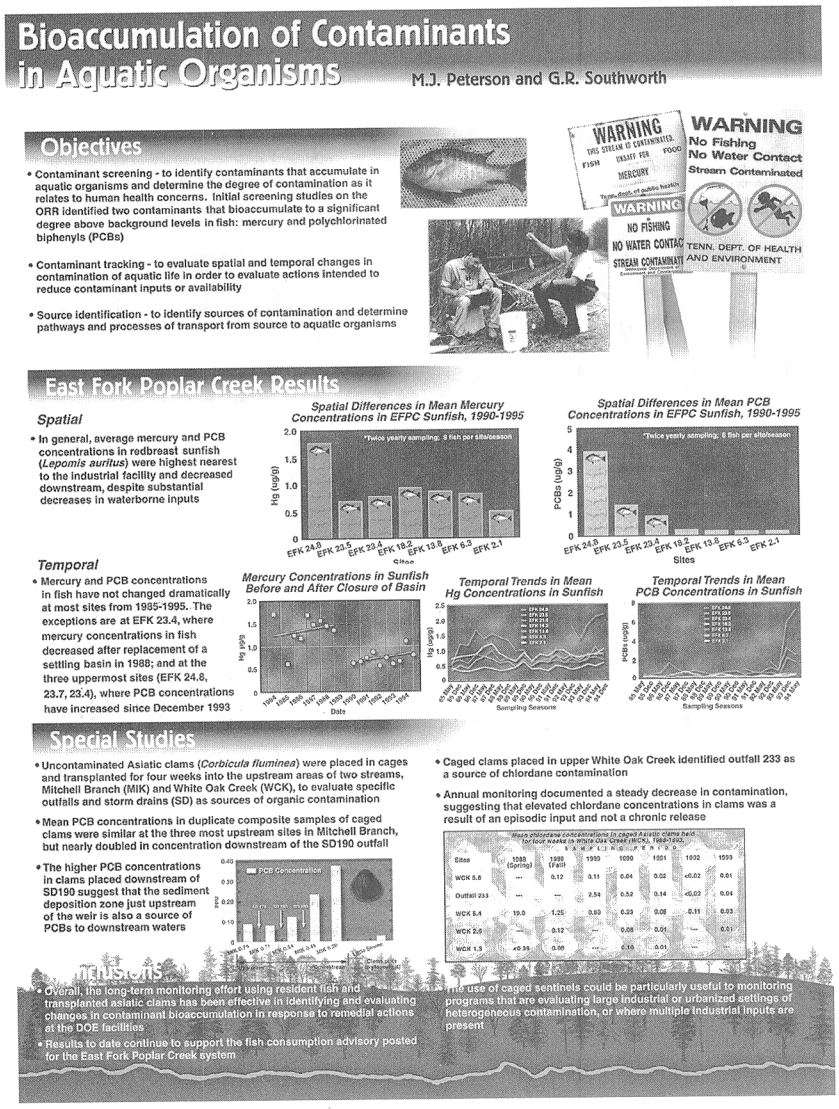


Figure 7.7 Bioaccumulation of contaminants in aquatic organisms

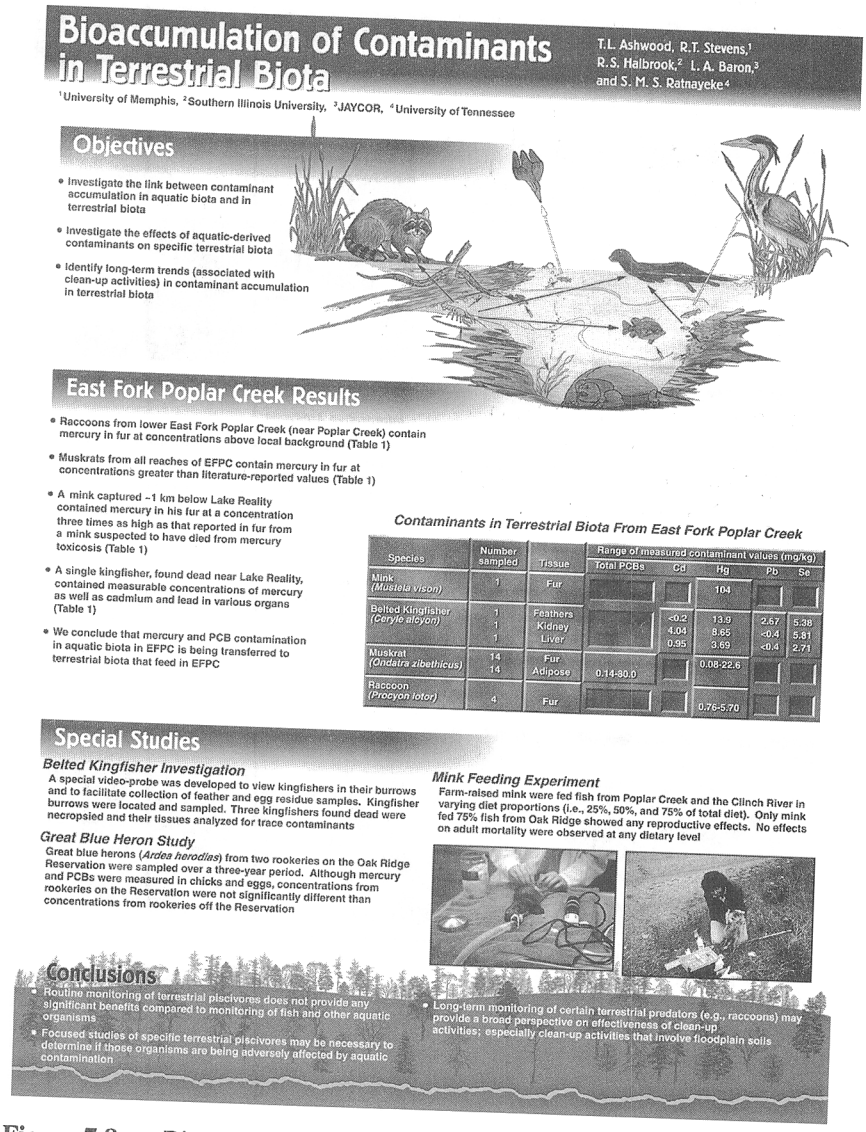


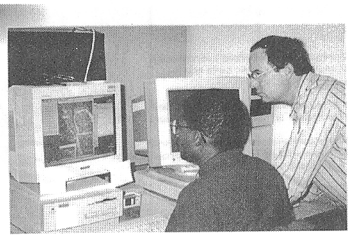
Figure 7.8 Bioaccumulation of contaminants in terrestrial biota

Integrative Approaches

S.W. Christensen, C.E. Duncan, R.A. Washington-Allen

Objectives

This is a long-term effort in which a variety of integrative approaches are used to provide interpretative analyses of the 10+ years of historical BMAP data. Two major efforts in this area are (1) achieving data integration by using the latest data management techniques, and (2) applying Geographic Information System (GIS) tools. These tasks are in varying stages of completion



Data Integration

Specific Objectives:

Construct an integrated BMAP data base to

- Share data with regulators (mandated)
- Support data needs of ecological risk assessors and BMAP Principal Investigators (PI's)
- Facilitate links with external data
- Enable broad, interpretive analyses

Process:

- A data model has been developed using Computer-Aided Software Engineering (CASE) tools
  - This involved extensive meetings with BMAP PI's
  - The data model is a kind of "common language" between the PI's and the users
- We implemented a relational data base in which the biological model augmented an existing data model developed for two other environmental sampling programs
- We are acquiring and loading data from multiple BMAP subject areas
- User-access tools and database "views" are being developed
- Selected data will be copied to the Oak Ridge Environmental Information System (OREIS) for widespread distribution

Interpretive Analyses:

The completed integrated data base will support broad interpretive analyses to address assessment and scientific questions, such as:

- To what extent can biotic patterns be explained by (1) natural temporal variability and (2) natural spatial variability?
- How can the relationships among biotic compartments be integrated with other factors?
- How can stronger inferences be drawn about causes of impact and responses to remediation?

Hardware/Software:

UNIX workstations with SAS, ORACLE, ARCVIEW, ARC/INFO, and ERDAS Imagine, available via Ethernet for X Window emulation on PCs

Geographic Information System



The 3-D model and animation were prepared by W. Hargrove, University of Tennessee. The data sources of the DEM and the orthophotograph are the Environmental Restoration Program's GISST/GCM/CPED/LMES Basemapping and Imagery Project and the OREIS.

- As an illustration of the use of GIS for data visualization (1) an orthophotograph of the upstream portion of East Fork Poplar Creek (EFPC) was combined with (2) a digital elevation model (DEM) of the same area. The result of the combination is (3) a 3-D model of EFPC
- The 3-D model was viewed from different angles and heights, using fly-through software in combination with movie-making software to render a short animated film on a Silicon Graphics Indigo workstation using the GIS GRASS 3-D module
- In the future, animations of creek flow, sediment concentration and deposition, as well as changes in stream species' habitat structure will be prepared from actual temporal data. The result will be a 4-D model or visualization of stream activity

Figure 7.9 Integrative approaches

CASE STUDY 6—BIOINDICATORS: EARLY SIGNS OF ENVIRONMENTAL DAMAGE AND RECOVERY

INTRODUCTION. Biological events, such as increases in the populations of pollution-sensitive species in a river or the return of a fish species to a once-polluted stream, can indicate recovery of an ecosystem. Such information helps scientists determine the extent to which remediation of a contaminated waterway has been successful.

Biological events that provide information about the environment include more, however, than increases and decreases in animal and plant species. A variety of pollutants resulting from human activities can interfere with the normal functioning of an organism, making it less able to grow normally or reproduce in its environment. Short of death, some of the more common but serious effects of environmental stressors on aquatic organisms are changes in behavior, growth, and reproduction. These changes in individual organisms, known as biological indicators (or bioindicators, for short), can eventually result in large-scale changes in biological communities and ecological systems.

Researchers in the Environmental Sciences Division (ESD) at ORNL are using wildlife species, such as fish and waterfowl, as bioindicators of ecosystem health. The goal of this research is to develop and improve the capabilities for detecting and predicting the effects of environmental stress on key, or "sentinel," ecological species. This information could lead to appropriate actions that prevent undesirable and irreversible effects in ecological systems.

Research to identify biomolecular, biochemical, physiological, and other organism-level responses of aquatic organisms to environmental stressors such as contaminants is ongoing. Oak Ridge National Laboratory researchers have developed bioindicators of pollutant stress and used these indicators to assess ecological health in creeks, reservoirs, rivers, and marine systems. Researchers have applied this approach to determining biological effects of contaminants in various waterways on or near the Oak Ridge Reservation: East Fork Poplar Creek, White Oak Creek, Mitchell Branch, the Clinch River, and Watts Bar Reservoir. Researchers studied bioindicators in the Pigeon River and in estuaries in South Carolina, all of which are polluted by pulp and paper mill effluents. Researchers have also looked at ecological effects in reservoirs in South Carolina and Georgia, which are contaminated by high levels of PCBs, and in natural lakes at Cape Cod, Massachusetts, which receive underground plumes of contaminants from a nearby military facility.

EARLY WARNINGS OF ENVIRONMENTAL DAMAGE. Bioindicators are useful because they are signs of impending environmental problems.

Early effects of pollution occur at the lower levels of biological organization. Changes in genes, cells, tissues, body chemical processes, and basic body functions appear before more severe disturbances occur in populations and ecosystems. These biochemical and molecular effects can be detected as changes in enzyme levels, the structure of cell membranes, and genetic material, or deoxyribonucleic acid (DNA). Changes at these subcellular levels induce a series of structural and functional responses at the next level of biological organization. For example, complex processes such as hormonal regulation, metabolism, and immune system responses can be impaired. These effects may eventually alter the organism's ability to grow, reproduce, or survive. All of these measurable changes serve as bioindicators of pollutant stress. Ultimately, irreversible and detrimental effects may be observed at the population and community level, adversely affecting the health of the ecosystem (Figure 7.10).

EXAMPLES OF BIOINDICATORS. Analysis of blood from a fish can indicate problems in organ function—a bioindicator. For example, the presence of specific types of enzymes (tranaminases) in fish blood can indicate liver impairment. Polychlorinated biphenyls and PAHs in the aquatic environment can inflict liver damage in fish, leading to more serious problems.

Elevated levels of another bioindicator, urea nitrogen, in fish blood reflect abnormalities in gill function or respiratory problems. When functioning normally, fish gills and kidneys excrete nitrogen-bearing waste products from the body. Fish gills can be damaged by heavy metals—mercury and aluminum—and acidified water. Researchers at ORNL have found evidence of such effects in fish from the Great South Fork Recreational Area in Tennessee and Kentucky. In this area, drainage from abandoned coal mines contributes both to high levels of heavy metals and to highly acidified surface waters. In the Great Smoky Mountains, acid rain can drastically raise the acidity level of mountain streams, periodically killing trout in hatcheries.

As these examples show, bioindicators of pollutant stress range from organ damage responses to increased mortality rates of organisms.

USES FOR BIOINDICATORS. The bioindicator approach is not the only method for monitoring possible ecological effects of pollutants. One traditional method is direct measurement of chemical pollutants in streams or in the organisms themselves. Another is the laboratory toxicity test in which the toxicity of a pollutant is determined by the death rate of exposed organisms. Bioindicators offer several types of information not available from other methods: (1) early warning of environmental damage; (2) the integrated effect of a variety of environmental stresses on the health of an organism and the population, community, and ecosystem; (3) relationships between the individual responses of exposed organisms to pollution and the effects at the population level; (4) early warning of potential harm to human health based on

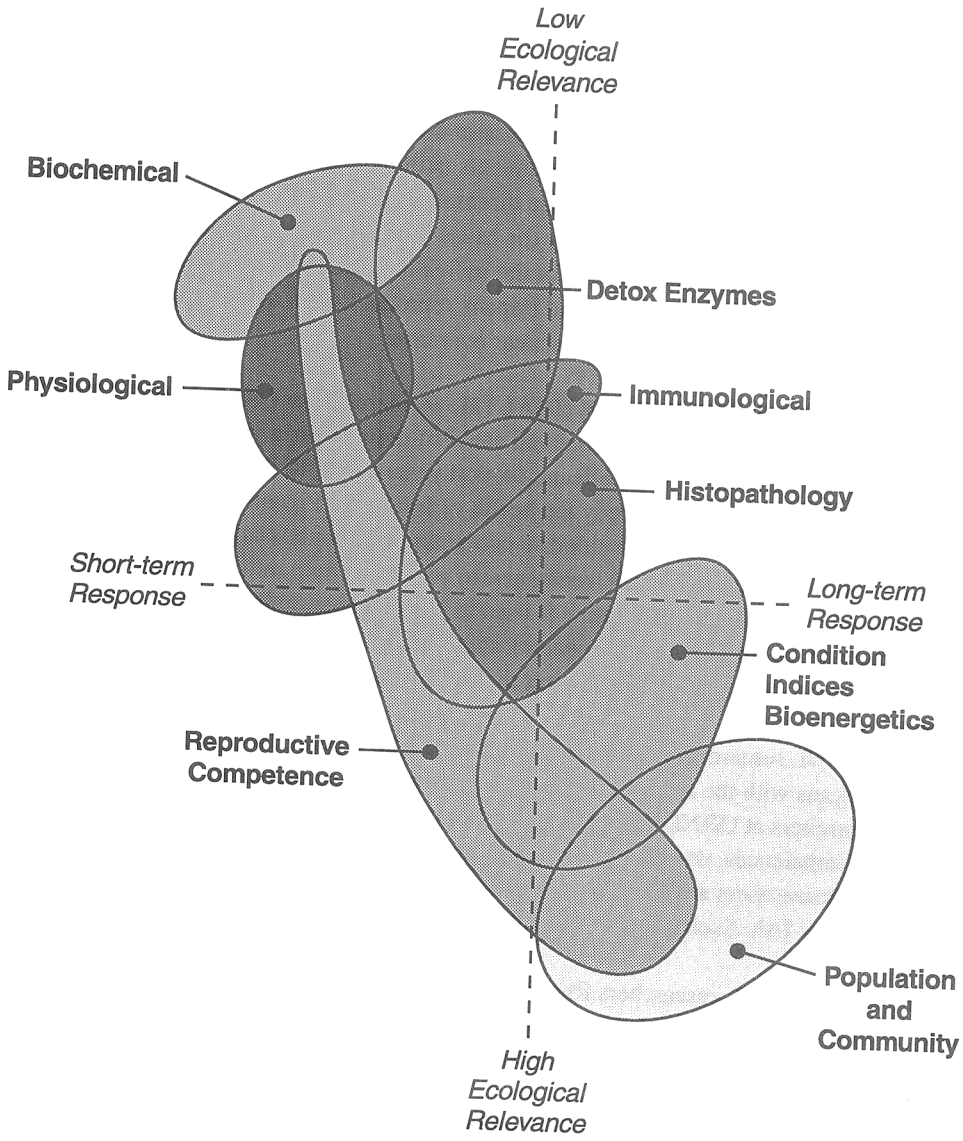


Figure 7.10 **Detectible changes in genes, cells, and tissues of an organism may later be the first signals of more severe disturbances in its population and community**

the responses of wildlife to pollution; and (5) the effectiveness of remediation efforts in decontaminating waterways.

The physiological condition of an animal reflects the combined effects of all of its environmental stresses such as contaminants, unfavorable temperature, suspended sediment, insufficient oxygen, and food shortages. Largemouth bass and striped bass—important sport fish commonly found in

East Tennessee reservoirs—are particularly good indicators of environmental stress because they are at the top of the aquatic food chain. Signs of impaired health in fish enable scientists to assess the nature and extent of environmental degradation.

Bioindicators can be used to establish cause-and-effect relationships between different levels of response to pollutants in individual organisms and for the entire population. For example, if a diseased fish is found in a stream, one should be able to determine not only the cause of the disease but also the potential consequences of that disease for the individual fish and for the population. If the disease kills young fish and impairs reproduction in older fish, the population may decline or even disappear over time.

BIOINDICATORS AND HUMAN HEALTH. Researchers at ORNL have found that bioindicators in the environment can also provide early warning signals of potential human health effects. Several years ago, ORNL researchers were asked by U.S. EPA to study the health of fish in the Pigeon River. This river was contaminated with chlorine-containing compounds called dioxins and other pollutants, primarily from a nearby paper and pulp mill. Dioxins are produced when elemental chlorine is used to bleach paper. Individuals downstream of the mill expressed concerns about its health effects, such as increased cancer rates, and filed a lawsuit that was eventually settled out of court.

When ORNL researchers examined sunfish from the Pigeon River, they found problems with the fishes' endocrine, reproductive, and metabolic systems. Researchers at ORNL detected altered hormone levels, metabolic and nutritional imbalances, and changes in the normal population and community dynamics. Researchers at ORNL also observed DNA damage and cancerous lesions in these fish. Such effects were attributed to chemicals released from the mill.

In addition, ORNL researchers found that individuals in the area could have been exposed to contaminants by eating fish and using water from the Pigeon River to irrigate crops and water livestock. Thus, some food sources may be contaminated with dioxins.

Is there a link between impaired fish health and a potential threat to human health? Recent studies show that dioxin and other environmental chemicals can disrupt the hormonal system by mimicking or inhibiting estrogens in organisms. Estrogens and other hormones are chemical messengers that regulate various processes in the body. Hormones act on parts of the body by sending chemical signals through receptors on or inside cells. This action can be blocked by chemicals that mimic a hormone and attach to its receptors. Because hormones regulate growth and reproduction, it is believed that hormone-disrupting chemicals could interfere with these processes, possibly leading to reproductive and developmental problems in humans.

Researchers at ORNL found evidence of altered sexual development—possible sex changes, impaired gonad development, and reduced steroid hormone concentrations—in fish taken from the upper and most contaminated section of the Pigeon River. Interestingly, breast cancer incidences in women are higher in the two counties downstream of the paper mill than in surrounding counties. There is only approximately a 1 in 500 probability that the observed incidences of breast cancer in these two counties are results of random chance alone. This example suggests that bioindicators could provide a reliable early warning that human health may be potentially endangered by increasing levels of environmental pollution. However, it is difficult to prove that effects to wildlife populations are attributable to certain suspect chemicals. It is even more difficult to prove that these chemicals are affecting humans. These difficulties can be addressed only through multidisciplinary research linking ecological, wildlife, human, and laboratory animal research and by building bridges among the human, veterinary, and environmental health sciences.

ENVIRONMENTAL RESTORATION. Since 1942, research and production activities on the Oak Ridge Reservation have resulted in occasional spills or discharges of hazardous materials, including radioactive cesium, PCBs, and mercury, to various waterways. Recent environmental laws and regulations such as the Clean Water Act, the Resource Conservation and Recovery Act, and the Comprehensive Environmental Response, Compensation, and Liability Act impose limits on releases of these materials and require that these materials be properly treated, recovered, and isolated from the environment. They also require cleaning up waterways and land contaminated by discharges of hazardous materials.

In the biological monitoring and abatement program at ESD (see Case Study 5), bioindicators are being used to evaluate the short- and long-term effectiveness of remedial actions on aquatic systems receiving discharges from ORNL, the Oak Ridge Y-12 plant, and the Oak Ridge K-25 site. These remedial actions are being taken to comply with environmental regulations. These assessments will guide regulators in determining the extent of environmental restoration required on the Oak Ridge Reservation. Additional bioindicator and contaminant data will be obtained later to determine the effectiveness of any reservation-wide cleanup.

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Acronyms

ANOVA	Analysis of variance
BC	Benchmark concentration
BMAP	Biological monitoring and abatement program
BOD	Biochemical oxygen demand
C_{18}	C_{18} octodecyl carbon (filtration column)
$CaCO_3$	Calcium carbonate
ChV	Chronic value
COC	Chemical of concern
COEC	Chemical of ecological concern
COPC	Chemical of potential concern
COPEC	Chemical of potential ecological concern
DMR	Discharge monitoring report
DNA	Deoxyribonucleic acid
EC	Effective concentration
EDTA	Ethylenediamine tetraacetate
EEC	Expected environmental concentration
EFPC	East Fork Poplar Creek
Eh	Redox-potential

EIA/EIS	Environmental impact assessment/statement
EMAP	Environmental Monitoring and Assessment Program (U.S. EPA)
ERA	Ecological risk assessment
ESD	Environmental Sciences Division (ORNL)
GC/MS	Gas chromatography/mass spectrophotometry
GMATC	Point estimate of MATC (based on a geometric mean)
GOES	Geostationary operational environmental satellite
H_2O	Water
HCl	Hydrochloric acid
HQ	Hazard quotient
IBI	Index of biotic integrity
IC	Inhibition concentration
KCl	Potassium chloride
LC	Lethal concentration
LOAEL	Lowest observed adverse effect level
LOEC	Lowest observed effect concentration
LOEL	Lowest observed effect level
MATC	Maximum acceptable toxicant concentration
$MgSO_4$	Magnesium sulfate
NaOH	Sodium hydroxide
NAWQA	National Water Quality Assessment Program
NEPA	National Environmental Policy Act
NH_3	Un-ionized ammonia

NH_4^+	Ionized ammonia
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOEL	No observed effect level
NPDES	National Pollutant Discharge Elimination System
OME	Ontario Ministry of the Environment
ORNL	Oak Ridge National Laboratory
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
ppt	Parts per thousand
QA/QC	Quality assurance/quality control
QAPP	Quality assurance project plan
SF	Safety factor
SIU	Significant industrial user
SOP	Standard operating procedure
TIE	Toxicity identification evaluation
TRE	Toxicity reduction evaluation
TU	Toxicity unit
U.S. DOE	U.S. Department of Energy
U.S. EPA	U.S. Environmental Protection Agency
U.S. FDA	U.S. Food and Drug Administration
USGS	U.S. Geological Survey
WER	Water-effects ratio

WET	Whole effluent toxicity (testing)
WPA	Watershed protection approach
WWTP	Wastewater treatment plant

Glossary

acute bioassay (test) Typically, a type of bioassay that uses an old, easy-to-handle, and hearty life stage of an organism to examine that organism's basic systemic or mortality responses over a relatively short bioassay's duration. The acute bioassay organism typically does not live out a significant portion of its life in the particular bioassay system. Consequently, acute bioassays can cost significantly less and be easier to conduct than related chronic bioassays. Acute bioassays can yield qualitative data that may suggest potential (effective or inhibitory) chronic effects. However, the LC data generated by acute tests can yield a range of mortality percentage-affected LC values (1 to 99%) by the use of statistical, regression-type programs such as Probit.

ambient Pertains to local or natural environments and surface waters.

anthropogenic Pertaining to human origins, human-caused effects, or effects resulting from human activities.

area (zone) of concern A physical area, habitat, or environment of many habitats that may be affected by anthropogenic activities and/or wastes.

artifactual toxicity Apparent toxicity that is not caused by water quality. Artifactual toxicity is a result of some type of technical error or improper manipulation of test materials or organisms.

bioaccumulation The act of concentrating a chemical present in the environment by a plant or animal. Bioaccumulation occurs by direct uptake from water and/or ingesting contaminated food.

biocriteria A measurement of ecological health that is based on comparison of plants and animals present in a particular environment to a "reference condition" in which human-made alterations are limited.

chemicals of concern/chemicals of ecological concern (COC/COEC) Lists of all chemicals that have been confirmed as existing within various media pathways that may allow exposure to humans (COC) or other biota (COEC)

within an area of concern. These confirmed lists also pertain to quantitative concentration data on chemicals in question and can also include confirmed toxicity effect data on humans (COC) or other biota (COEC). Typically, these lists are smaller than potential chemical lists (COPC/COPEC).

chemicals of potential concern/chemicals of potential ecological concern (COPC/COPEC) Lists of all chemicals that, historically, may have existed at specific sites (COC) or habitats (COEC) within an area of concern. Classically, the COPC list is used to represent those chemicals that pose potential health risks to humans, and the COPEC list is used to represent those chemicals that pose potential ecological health risks to other biota. In many instances, these initial lists can contain the same chemicals, for such lists are derived from qualitative data.

chronic bioassays (tests) Typically, a type of bioassay that uses a relatively young, hard-to-handle, and sensitive life stage of an organism to assess a wide range of that organism's sublethal and lethal responses over a relatively long bioassay's duration. The young chronic bioassay organism can often grow, develop, reproduce, and live out a significant portion of its life in the particular bioassay system. Chronic tests, then, yield quantitative data that are related to the EC/IC effects as well as LC or mortality data. Such chronic data can yield both NOEC, GMATC, and LOEC data as well as ranges for percentage-affected (1 to 99%) EC, IC, and LC data by the use of ANOVA (Dunnett's test) and regression-type (Probit) statistical programs.

chronic value (ChV) The geometric mean of the LOEC and the NOEC (now known as the MATC).

discharge monitoring report quality assurance (DMR QA) samples A program sponsored by U.S. EPA to monitor biomonitoring laboratory proficiency. Unknown samples are sent to participating laboratories. Laboratories run the samples on the types of tests that they typically run. The laboratory's results are compared to ranges of acceptable performance. A laboratory's certification status may be linked to its performance on these samples.

ecological/risk analysis Determination of the probability of adverse effects on biota resulting from an environmental hazard (a chemical, physical, or biological agent occurring in or mediated by the environment).

ecological/risk assessments The process of defining and quantifying risks to biota and determining the acceptability of those risks (note: ecological implies nonhuman).

ecological/risk management The process of deciding what actions to take in response to a risk, based on the evaluation of that risk with assessments and analysis.

ecoregion An area of relative homogeneity based on one or more attributes in ecological systems.

effective concentration (EC) The concentration of a toxicant or dilution of an effluent that will cause a certain measurable effect (such as lack of weight gain) to a certain proportion of the test population. An EC_{50}, for example, is the concentration that will cause 50% of the test population to not gain weight.

fetax Frog embryo teratogenesis assay using *Xenopus* species. A new type of bioassay that uses frog embryos as the test organism.

flow-through bioassay (test) A bioassay in which the sample consists of a continuous stream of test solution that flows through the test system at a constant rate during the test's duration.

habitat assessment Assessment that determines the suitability of ecosystems as habitat for a species.

hazard assessment/analysis The determination of a hazard. In predictive risk assessments, hazard assessment/analysis is a preliminary activity that helps to define assessment endpoints by determining which environmental components are potentially exposed to toxic concentrations and in what ways they might be affected. An alternative assessment method determines if a hazard exists by comparing the magnitudes of expected environmental concentrations to toxicological test endpoints for a contaminant.

hazard quotient A hazard quotient (HQ) is used in ERAs. An HQ value, calculated for a specific COPC/COPEC, is the ratio of the upper 95% confidence limit for the chemical's EEC divided by the BC. The BC is the ecological toxicity value for the chemical being assessed and is typically based on NOEC, GMATC, or any justifiable (best available) chronic or acute toxicity values. This ratio determines whether the COPC/COPEC becomes a COC/COEC. When the HQ is greater than 1.0, then the chemical's environmental concentration exceeds its presumed safe concentration value (BC) and the chemical would pose a potential threat to the health of receptor organisms (human or otherwise). Many ecotoxicologists often consider chemicals that have an HQ of greater than 0.3 as also posing an environmental risk, especially to nonhuman biota. This is a conservative criterion that is used because such chemicals may contribute to chronic effects because of their synergistic effects on the receptors.

indicator species An organism that represents, ecologically and taxonomically, those major taxonomic groups or *foraging guilds* of receptor organisms that would be located within the area of concern for risk assessment purposes. Such an indicator species itself need not live within the area of concern for its bioassay data to be useful.

inhibition concentration (IC) The concentration of a toxicant or the dilution of an effluent that will cause inhibition of an effect such as reproduction. An IC_{25}, for example, is the concentration that will cause a 25% reduction in reproduction for a test group.

lethal concentration (LC) The concentration of a toxicant or the dilution of an effluent that will cause mortality to a certain percentage of the test population. An LC_{50}, for example, is the concentration that will kill 50% of the test organisms.

liquid-media bioassays A class of bioassays that involves the use of some type of liquid media in which bioassay organisms live for the duration of the bioassays. Some common liquid media for estuarine and marine bioassays include liquid-media-stressor concentrations, in a geometric series, specific for a particular bioassay; whole wastewater effluent or its treatment dilutions; laboratory-prepared water; ambient water (locally unaffected water); and receiving water (water within the area of concern).

liquid-phase sediment bioassays Liquid-phase sediment bioassays are similar to liquid-media bioassays, except that the liquid-phase test medium is an actual liquid fraction, or extract made from mixing sediments, sludges, soils, or biosolids with any of the common liquid media described above. Sometimes, depending on the bioassay and organisms used, such extracts are either used as is or are filtered before use.

lowest observed effect concentration/lowest observed adverse effect level (LOEC/LOAEL) The lowest treatment concentration in a test that is significantly different (in test species' response) from that seen in the control treatment (control species' response).

maximum acceptable toxicant concentration (MATC) The theoretical concentration point, between the NOEC (NOEL) and the LOEC (LOEL), at which a bioassay organism's adversely significant responses would start to appear. The point estimation of the MATC is the GMATC or the geometric mean of the NOEC and LOEC data (this was formerly called the "chronic value," or ChV).

media pathways All possible routes by which a receptor species can be exposed to all possible sources of adverse affects. Each medium pathway

conveys a potential ecological risk to the receptor species based on the receptor's relative degree of exposure to each medium. Exposure occurs by ingestion of, physical contact with, or a close association to a medium. The collective ecological risk, from all media pathways, to the receptor is assessed by adding a receptor's particular degree of risk from each separate medium. The common media for a receptor species are other closely associating receptors, a receptor's food, surface water, groundwater, sediment, soil, and air.

mesocosm bioassay A mesocosm bioassay is a multispecies, controlled ecosystem designed to address potential ecological effects from anthropogenic sources and to take into account the between-species effects that would not be determined by using bioassay tests on separate species.

negative control A negative control can be used in any bioassay. In sediment bioassays, the negative control consists of uncontaminated sediment that is acceptable to the test species. If a solvent is used to dissolve some toxicant or mixture into a chosen test medium (water or sediment), then the negative control would have the same solvent concentration as is used in test treatments.

no observed effect concentration/no observed adverse effect level (NOEC/NOAEL) The highest treatment concentration in a test that is not significantly different (in test species' response) from that seen in the control treatment (control species' response).

receptor species A resident organism that lives within the area of concern and, for the purposes of risk assessment, is presumed to be subject to potential adverse effects arising from anthropogenic activities or wastes affecting that area of concern.

reference control A reference control can be used in any bioassay. In sediment bioassays, the reference sediment control may be the same material as that of the negative control, should be uncontaminated, and is typically sediment that also comes from an area near that of the sampled (unknown) sediments. The reference sediment should have the same qualities as the sample sediment (grain size, water content, Eh, pH, interstitial water salinity, total organic carbon content, and other significant characteristics).

reference site A water body that has one of the best habitats and aquatic communities available in a particular geographic area. Information from a reference site is used to develop biocriteria.

reference toxicity tests Tests designed to determine whether test organisms used in a biomonitoring test were normal and healthy at the time of the testing. Animals are placed in varying concentrations of known reference toxicants, and effects such as mortality are noted. The response of the animals is

compared to previously determined normal ranges. For the results of a biomonitoring test to be considered valid, the animals must respond normally (show effects as predicted).

safety factor (SF) A safety factor is a numerical value that, in effect, adjusts the original IC, EC, or LC affected data value to a level that could be professionally judged as similar to an NOEC/NOAEL or no effect level if an actual bioassay to determine the NOEC/NOAEL has been done.

sample holding time Sample holding times are set according to standards. The shorter the time period, the more likely it is that a sample will not change chemically or biologically from its collected state. Typically, samples are cooled to temperatures to effectively slow down any chemical/biological activity within the sample's matrix. Exceeding the standard sample holding time for a particular test can invalidate the test's results.

sediment bioassays A class of bioassays that involve the use of some type of sediment, wet soil, or biosolids and use bioassay organisms that live on, within, or closely associated with such particulate matter for the duration of the bioassays. There are two types of sediment bioassays: liquid phase and solid phase.

solid-phase (whole) sediment bioassays Solid-phase sediment tests are concerned with providing toxicity data on organisms that live within or on, or are closely associated with, sediments. These bioassays use the contaminated sediment sample itself and/or a series of percent-sediment concentrations derived by combining the control and/or reference sediments with contaminated sediments according to test-specific methods.

static bioassay (test) A bioassay in which the test solution (sample) is not changed for the duration of the test.

static-renewal bioassay (test) A bioassay in which each sample is changed, all at once, with a new sample, preferably every 24 hours, until the test ends.

static-renewal-flow bioassay (test) A bioassay in which each sample is changed with a new sample by completely pumping each new sample through the test at a constant rate, preferably within a 24-hour period, for the duration of the test.

stressor An anthropogenic activity, chemical, ecological relationship, or similar agent that would not typically be part of a particular ecosystem and would serve to change the existing homeostasis within a particular ecosystem as a result of the agent's sudden appearance. This term typically refers to the source or catalyst that causes a significant effect on a particular ecological

environment. Bioassays use this term to mean an external source of stress that may cause measurable adverse effects on a bioassay organism within an artificially controlled bioassay system so that a degree of stress within an ecosystem can be estimated by using conditions common to both the bioassay system and the ecosystem.

target receptor An organism that resides within the area of concern and serves the same purpose for risk assessment as the indicator species but is also cited adequately in the ecotoxicological literature as a commonly used bioassay organism.

teratogenic Causing developmental deformities. The effects are most pronounced during the early developmental stages of an organism's life.

toxicity identification evaluation (TIE) A systematic three-phase process to characterize, identify, and confirm the toxicant(s) responsible for a wastewater toxicity problem. The TIE is a tool used in the development of a toxicity reduction evaluation.

toxicity reduction evaluation (TRE) An investigation to identify the causes of a toxicity problem and to develop and implement a plan to reduce or eliminate the problem.

toxicity unit (TU) A ratio used to compare toxicity data from different bioassays and bioassay organisms. A chronic TU is the ratio of 100 divided by the GMATC of a chronic bioassay. An acute TU is the ratio of 100 divided by the LC_{50} (typically) of an acute bioassay.

water quality criterion A particular concentration of a chemical thought to be protective of a beneficial use of a water body. A criterion has no legal status but is often the basis for a standard.

water quality standard A concentration of a chemical that has legal status when used by a regulatory agency. A standard is based on both a criterion and a use that must be protected.

watershed An area of land draining to a specific point on a stream, lake, or other receiving body of water (also known as a drainage basin).

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