188

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EPA

# Biological Criteria

*National Program Guidance For Surface Waters*

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# *APPENDIX C*

***Biological Criteria:***
***National Program Guidance***
***for Surface Waters***

**APPENDIX C**

WATER QUALITY STANDARDS HANDBOOK

SECOND EDITION

# *Biological Criteria*

## *National Program Guidance for Surface Waters*

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U. S. Environmental Protection Agency
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# Contents

## Part II: The Implementation Process

# *Acknowledgments*

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*Special recognition goes to the Steering Committee who helped develop document goals and made a significant contribution toward the final guidance. Members of the Steering Committee include:*

| | |
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*Editor*

*In Memory of*

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18585

# Definitions

To effectively use biological criteria, a clear understanding of how these criteria are developed and applied in a water quality standards framework is necessary. This requires, in part, that users of biological criteria start from the same frame of reference. To help form this frame of reference, the following definitions are provided. Please consider them carefully to ensure a consistent interpretation of this document.

## Definitions

- An **AQUATIC COMMUNITY** is an association of interacting populations of aquatic organisms in a given waterbody or habitat.
- A **BIOLOGICAL ASSESSMENT** is an evaluation of the biological condition of a waterbody using biological surveys and other direct measurements of resident biota in surface waters.
- **BIOLOGICAL CRITERIA**, or biocriteria, are numerical values or narrative expressions that describe the reference biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use.
- **BIOLOGICAL INTEGRITY** is functionally defined as the condition of the aquatic community inhabiting unimpaired waterbodies of a specified habitat as measured by community structure and function.
- **BIOLOGICAL MONITORING** is the use of a biological entity as a detector and its response as a measure to determine environmental conditions. Toxicity tests and biological surveys are common biomonitoring methods.
- A **BIOLOGICAL SURVEY**, or biosurvey, consists of collecting, processing and analyzing representative portions of a resident aquatic community to determine the community structure and function.
- A **COMMUNITY COMPONENT** is any portion of a biological community. The community component may pertain to the taxomonic group (fish, invertebrates, algae), the taxonomic category (phylum, order, family, genus, species), the feeding strategy (herbivore, omnivore, carnivore) or organizational level (individual, population, community association) of a biological entity within the aquatic community.
- **REGIONS OF ECOLOGICAL SIMILARITY** describe a relatively homogeneous area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, or other ecologically relevant variable. Regions of ecological similarity help define the potential for designated use classifications of specific waterbodies.
- **DESIGNATED USES** are those uses specified in water quality standards for each waterbody or segment whether or not they are being attained.
- An **IMPACT** is a change in the chemical, physical or biological quality or condition of a waterbody caused by external sources.
- An **IMPAIRMENT** is a detrimental effect on the biological integrity of a waterbody caused by an impact that prevents attainment of the designated use.
- A **POPULATION** is an aggregate of interbreeding individuals of a biological species within a specified location.
- A **WATER QUALITY ASSESSMENT** is an evaluation of the condition of a waterbody using biological surveys, chemical-specific analyses of pollutants in waterbodies, and toxicity tests.
- An **ECOLOGICAL ASSESSMENT** is an evaluation of the condition of a waterbody using water quality and physical habitat assessment methods.

# *Executive Summary*

The Clean Water Act (Act) directs the U.S. Environmental Protection Agency (EPA) to develop programs that will evaluate, restore and maintain the chemical, physical, and biological integrity of the Nation's waters. In response to this directive, States and EPA implemented chemically based water quality programs that successfully addressed significant water pollution problems. However, these programs alone cannot identify or address all surface water pollution problems. To create a more comprehensive program, EPA is setting a new priority for the development of biological water quality criteria. The initial phase of this program directs State adoption of narrative biological criteria as part of State water quality standards. This effort will help States and EPA achieve the objectives of the Clean Water Act set forth in Section 101 and comply with statutory requirements under Sections 303 and 304. The *Water Quality Standards Regulation* provides additional authority for biological criteria development.

In accordance with priorities established in the *FY 1991 Agency Operating Guidance*, States are to adopt narrative biological criteria into State water quality standards during the FY 1991-1993 triennium. To support this priority, EPA is developing a *Policy on the Use of Biological Assessments and Criteria in the Water Quality Program* and is providing this program guidance document on biological criteria.

This document provides guidance for development and implementation of narrative biological criteria. Future guidance documents will provide additional technical information to facilitate development and implementation of narrative and numeric criteria for each of the surface water types.

When implemented, biological criteria will expand and improve water quality standards programs, help identify impairment of beneficial uses, and help set program priorities. Biological criteria are valuable because they directly measure the condition of the resource at risk, detect problems that other methods may miss or underestimate, and provide a systematic process for measuring progress resulting from the implementation of water quality programs.

Biological criteria require direct measurements of the structure and function of resident aquatic communities to determine biological integrity and ecological function. They supplement, rather than replace chemical and toxicological methods. It is EPA's policy that biological survey methods be fully integrated with toxicity and chemical-specific assessment methods and that chemical-specific criteria, whole-effluent toxicity evaluations and biological criteria be used as independent evaluations of non-attainment of designated uses.

Biological criteria are narrative expressions or numerical values that describe the biological integrity of aquatic communities inhabiting waters of a given aquatic life use. They are developed under the assumptions that surface waters impacted by antnropogenic activities may contain impaired aquatic communities (the greater the impact the greater the expected impairment) and that surface waters not impacted by anthropogenic activities are generally not impaired. Measures of aquatic community structure and function in unimpaired surface waters functionally define biological integrity and form the basis for establishing the biological criteria.

Narrative biological criteria are definable statements of condition or attainable goals for a given use designation. They establish a positive statement about aquatic community characteristics expected to occur within a waterbody (e.g., "Aquatic life shall be as it naturally occurs" or "A natural variety of aquatic life shall be present and all functional groups well represented"). These criteria can be developed using existing information. Numeric criteria describe the expected attainable community attributes and establish values based on measures such as species richness, presence or absence of indicator taxa, and distribution of classes of organisms. To implement narrative criteria and develop numeric criteria, biota in reference waters must be carefully assessed. These are used as the reference values to determine if, and to what extent, an impacted surface waterbody is impaired.

Biological criteria support designated aquatic life use classifications for application in standards. The designated use determines the benefit or purpose to be derived from the waterbody; the criteria provide a measure to determine if the use is impaired. Refinement of State water quality standards to include more detailed language about aquatic life is essential to fully implement a biological criteria program. Data collected from biosurveys can identify consistently distinct characteristics among aquatic communities inhabiting different waters with the same designated use. These biological and ecological characteristics may be used to define separate categories within a designated use, or separate one designated use into two or more use classifications.

To develop values for biological criteria, States should (1) identify unimpaired reference waterbodies to establish the reference condition and (2) characterize the aquatic communities inhabiting reference surface waters. Currently, two principal approaches are used to establish reference sites: (1) the site-specific approach, which may require upstream-downstream or near field-far field evaluations, and (2) the regional approach, which identifies similarities in the physico-chemical characteristics of watersheds that influence aquatic ecology. The basis for choosing reference sites depends on classifying the habitat type and locating unimpaired (minimally impacted) waters.

Once reference sites are selected, their biological integrity must be evaluated using quantifiable biological surveys. The success of the survey will depend in part on the careful selection of aquatic community components (e.g., fish, macroinvertebrates, algae). These components should serve as effective indicators of high biological integrity, represent a range of pollution tolerances, provide predictable, repeatable results, and be readily identified by trained State personnel. Well-planned quality assurance protocols are required to reduce variability in data collection and to assess the natural variability inherent in aquatic communities. A quality survey will include multiple community components and may be measured using a variety of metrics. Since multiple approaches are available, factors to consider when choosing possible approaches for assessing biological integrity are presented in this document and will be further developed in future technical guidance documents.

To apply biological criteria in a water quality standards program, standardized sampling methods and statistical protocols must be used. These procedures must be sensitive enough to identify significant differences between established criteria and tested communities. There are three possible outcomes from hypothesis testing using these analyses: (1) the use is impaired, (2) the biological criteria are met, or (3) the outcome is indeterminate. If the use is impaired, efforts to diagnose the cause(s) will help determine appropriate action. If the use is not impaired, no action is required based on these analyses. The outcome will be indeterminate if the study design or evaluation was incomplete. In this case, States would need to re-evaluate their protocols.

If the designated use is impaired, diagnosis is the next step. During diagnostic evaluations three main impact categories must be considered: chemical, physical, and biological stress. Two questions are posed during initial diagnosis: (1) what are obvious potential causes of impairment, and (2) what possible causes do the biological data suggest? Obvious potential causes of impairment are often identified during normal field biological assessments. When an impaired use cannot be easily related to an obvious cause, the diagnostic process becomes investigative and iterative. Normally the diagnoses of biological impairments are relatively straightforward; States can use biological criteria to confirm impairment from a known source of impact.

There is considerable State interest in integrating biological assessments and criteria in water quality management programs. A minimum of 20 States now use some form of standardized biological assessments to determine the status of biota in State waters. Of these, 15 States are developing biological assessments for future criteria development. Five States use biological criteria to define aquatic life use classifications and to enforce water quality standards. Several States have established narrative biological criteria in their standards. One State has instituted numeric biological criteria.

Whether a State is just beginning to establish narrative biological criteria or is developing a fully integrated biological approach, the programmatic expansion from source control to resource management represents a natural progression in water quality programs. Implementation of biological criteria will provide new options for expanding the scope and application of ecological perspectives.

# Part I

# *Program Elements*

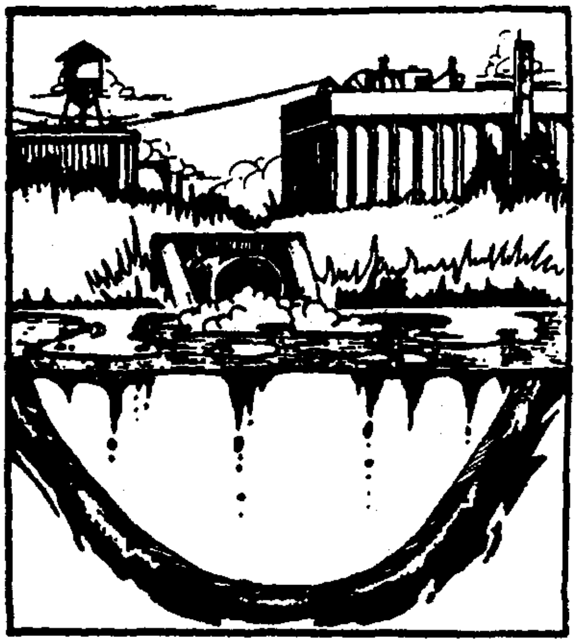
# Chapter 1

# *Introduction*

The principal objectives of the Clean Water Act are "to restore and maintain the chemical, physical and biological integrity of the Nation's waters" (Section 101). To achieve these objectives, EPA, States, the regulated community, and the public need comprehensive information about the ecological integrity of aquatic environments. Such information will help us identify waters requiring special protection and those that will benefit most from regulatory efforts.

To meet the objectives of the Act and to comply with statutory requirements under Sections 303 and 304, States are to adopt biological criteria in State standards. The *Water Quality Standards Regulation* provides additional authority for this effort. In accordance with the *FY 1991 Agency Operating Guidance*, States and qualified Indian tribes are to adopt narrative biological criteria into State water quality standards during the FY 1991-1993 triennium. To support this effort, EPA is developing a *Policy on the Use of Biological Assessments and Criteria in the Water Quality Program* and providing this program guidance document on biological criteria.

Like other water quality criteria, biological criteria identify water quality impairments, support regulatory controls that address water quality problems, and assess improvements in water quality from regulatory efforts. Biological criteria are numerical values or narrative expressions that describe the reference biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use. They are developed through the direct measurement of aquatic community components inhabiting unimpaired surface waters.



*Anthropogenic impacts, including point source discharges, nonpoint runoff, and habitat degradation continue to impair the nation's surface waters.*

Biological criteria complement current programs. Of the three objectives identified in the Act (chemical, physical, and biological integrity), current water quality programs focus on direct measures of chemical integrity (chemical-specific and whole-effluent toxicity) and, to some degree, physical integrity through several conventional criteria (e.g., pH, turbidity, dissolved oxygen). Implementation of these programs has significantly improved water quality. However, as we learn more about aquatic ecosystems it is apparent that other sources of waterbody impairment exist. Biological impairments from diffuse sources and habitat degradation can be greater than those caused by point source discharges (Judy et al. 1987; Miller et al. 1989). In Ohio, evaluation of instream biota indicated that 36 percent of impaired stream segments could not be detected using chemical criteria alone (see Fig. 1). Although effective for their purpose, chemical-specific criteria and whole-effluent toxicity provide only indirect evaluations and protection of biological integrity (see Table 1).

To effectively address our remaining water quality problems we need to develop more integrated and comprehensive evaluations. Chemical and physical integrity are necessary, but not sufficient conditions to attain biological integrity, and only when chemical, physical, and biological integrity are achieved, is ecological integrity possible (see Fig. 2). Biological criteria provide an essential third element for water quality management and serve as a natural progression in regulatory programs. Incorporating biological criteria into a fully integrated program directly protects the biological integrity of surface waters and provides indirect protection for chemical and physical integrity (see Table 2). Chemical-specific criteria, whole-effluent toxicity evaluations, and biological criteria, when used together, complement the relative strengths and weaknesses of each approach.

**Figure 1.—Ohio Biosurvey Results Agree with Instream Chemistry or Reveal Unknown Problems**

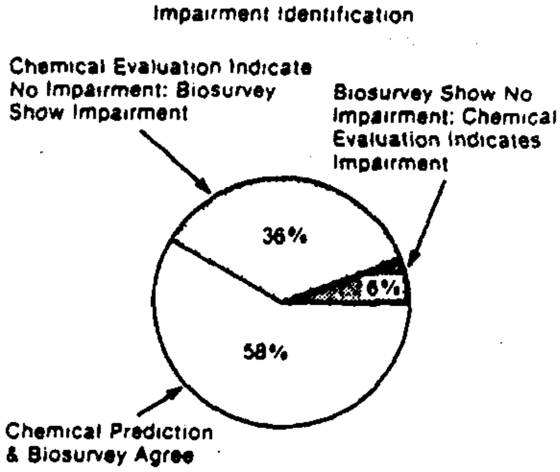



Fig. 1. In an intensive survey, 431 sites in Ohio were assessed using instream chemistry and biological surveys. In 36% of the cases, chemical evaluations implied no impairment but biological survey evaluations showed impairment. In 58% of the cases the chemical and biological assessments agreed. Of these, 17% identified waters with no impairment, 41% identified waters which were considered impaired. (Modified from Ohio EPA Water Quality Inventory, 1988.)

Biological assessments have been used in biomonitoring programs by States for many years. In this respect, biological criteria support earlier work. However, implementing biological criteria in water quality standards provides a systematic, structured, and objective process for making decisions about compliance with water quality standards. This distinguishes biological criteria from earlier use of biological information and increases the value of biological data in regulatory programs.

**Table 1.—Current Water Quality Program Protection of the Three Elements of Ecological Integrity.**

| ELEMENTS OF ECOLOGICAL INTEGRITY | PROGRAM THAT DIRECTLY PROTECTS | PROGRAM THAT INDIRECTLY PROTECTS |
|---|---|---|
| Chemical Integrity | Chemical Specific Criteria (toxics)<br>Whole Effluent Toxicity (toxics) | |
| Physical Integrity | Criteria for Conventionals (pH, DO, turbidity) | |
| Biological Integrity | | Chemical-Whole Effluent Toxicity (biotic response in lab) |

Table 1. Current programs focus on chemical specific and whole-effluent toxicity evaluations. Both are valuable approaches for the direct evaluation and protection of chemical integrity. Physical integrity is also directly protected to a limited degree through criteria for conventional pollutants. Biological integrity is only indirectly protected under the assumption that by evaluating toxicity to organisms in laboratory studies estimates can be made about the toxicity to other organisms inhabiting ambient waters.

**Table 2.—Water Quality Programs that Incorporate Biological Criteria to Protect Elements of Ecological Integrity.**

| ELEMENTS OF ECOLOGICAL INTEGRITY | DIRECTLY PROTECTS | INDIRECTLY PROTECTS |
|---|---|---|
| Chemical Integrity | Chemical Specific Criteria (toxics) Whole Effluent Toxicity (toxics) | Biocriteria (identification of impairment) |
| Physical Integrity | Criteria for conventionals (pH, temp., DO) | Biocriteria (habitat evaluation) |
| Biological Integrity | Biocriteria (biotic response in surface water) | Chemical-Whole Effluent Testing (biotic response in lab) |

Table 2 When biological criteria are incorporated into water quality programs the biological integrity of surface waters may be directly evaluated and protected. Biological criteria also provide additional benefits by requiring an evaluation of physical integrity and providing a monitoring tool to assess the effectiveness of current chemically based criteria.

**Figure 2.—The Elements of Ecological Integrity**

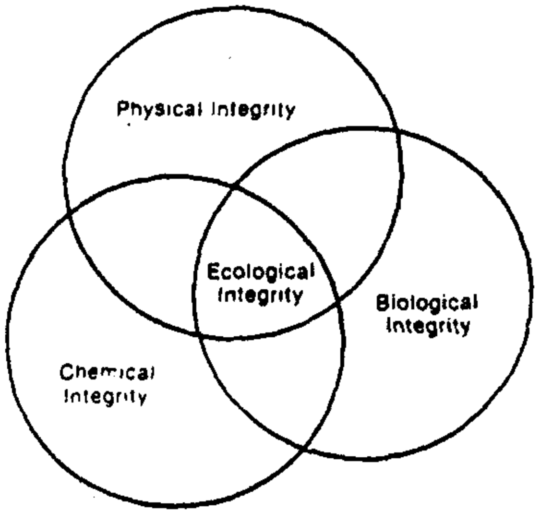



Fig 2: Ecological Integrity is attainable when chemical, physical, and biological integrity occur simultaneously.

## Value of Biological Criteria

Biological criteria provide an effective tool for addressing remaining water quality problems by directing regulatory efforts toward assessing the biological resources at risk from chemical, physical or biological impacts. A primary strength of biological criteria is the detection of water quality problems that other methods may miss or underestimate. Biological criteria can be used to determine to what extent current regulations are protecting the use.

Biological assessments provide integrated evaluations of water quality. They can identify impairments from contamination of the water column and sediments from unknown or unregulated chemicals, non-chemical impacts, and altered physical habitat. Resident biota function as continual monitors of environmental quality, increasing the likelihood of detecting the effects of episodic events (e.g., spills, dumping, treatment plant malfunctions, nutrient enrichment), toxic nonpoint source pollution (e.g., agricultural pesticides), cumulative pollution (i.e., multiple impacts over time or continuous low-level stress), or other impacts that periodic chemical sampling is unlikely to detect. Impacts on the physical habitat such as sedimentation from stormwater runoff and the effects of physical or structural habitat alterations (e.g., dredging, filling, channelization) can also be detected.

Biological criteria require the direct measure of resident aquatic community structure and function to determine biological integrity and ecological function. Using these measures, impairment can be detected and evaluated without knowing the impact(s) that may cause the impairment.

Biological criteria provide a regulatory framework for addressing water quality problems and offer additional benefits, including providing:

- the basis for characterizing high quality waters and identifying habitats and community components requiring special protection under State anti-degradation policies;
- a framework for deciding 319 actions for best control of nonpoint source pollution;
- an evaluation of surface water impairments predicted by chemical analyses, toxicity

testing, and fate and transport modeling (e.g., wasteload allocation);

- improvements in water quality standards (including refinement of use classifications);
- a process for demonstrating improvements in water quality after implementation of pollution controls;
- additional diagnostic tools.

The role of biological criteria as a regulatory tool is being realized in some States (e.g., Arkansas, Maine, Ohio, North Carolina, Vermont). Biological assessments and criteria have been useful for regulatory, resource protection, and monitoring and reporting programs. By incorporating biological criteria in programs, States can improve standards setting and enforcement, measure impairments from permit violations, and refine wasteload allocation models. In addition, the location, extent, and type of biological impairments measured in a waterbody provide valuable information needed for identifying the cause of impairment and determining actions required to improve water quality. Biological assessment and criteria programs provide a cost-effective method for evaluating water quality when a standardized, systematic approach to study design, field methods, and data analysis is established (Ohio EPA 1988a).

# Process for Implementation

The implementation of biological criteria will follow the same process used for current chemical-specific and whole-effluent toxicity applications: national guidance produced by U.S. EPA will support States working to establish State standards for the implementation of regulatory programs (see Table 3). Biological criteria differ, however, in the degree of State involvement required. Because surface waters vary significantly from region to region, EPA will provide guidance on acceptable approaches for biological criteria development rather than specific criteria with numerical limitations. States are to establish assessment procedures, conduct field evaluations, and determine criteria values to implement biological criteria in State standards and apply them in regulatory programs.

The degree of State involvement required influences how biological criteria will be implemented. It is expected that States will implement these criteria in phases.

- **Phase I** includes the development and adoption of narrative biological criteria into State standards for all surface waters (streams, rivers, lakes, wetlands, estuaries). Definitions of terms and expressions in the narratives must be included in these standards (see the Narrative Criteria Section, Chapter 3). Adoption of narrative biological criteria in State standards provides the legal and programmatic basis for using ambient biological surveys and assessments in regulatory actions

- **Phase II** includes the development of an implementation plan. The plan should include program objectives, study design, research protocols, criteria for selecting reference conditions and community components, quality assurance and quality control procedures.

**Table 3.—Process for Implementation of Water Quality Standards.**

| CRITERIA | EPA GUIDANCE | STATE IMPLEMENTATION | STATE APPLICATION |
|---|---|---|---|
| Chemical Specific | Pollutant specific numeric criteria | State Standards<br>· use designation<br>· numeric criteria<br>· antidegradation | Permit limits Monitoring<br>Best Management Practices<br>Wasteload allocation |
| Narrative Free Forms | Whole effluent toxicity guidance | Water Quality Narrative<br>· no toxic amounts translator | Permit limits Monitoring<br>Wasteload allocation<br>Best Management Practices |
| Biological | Biosurvey minimum requirement guidance | State Standards<br>· refined use<br>· narrative/numeric criteria<br>· antidegradation | Permit conditions Monitoring<br>Best Management Practices<br>Wasteload allocation |

Table 3 Similar to chemical specific criteria and whole effluent toxicity evaluations. EPA is providing guidance to States for the adoption of biological criteria into State standards to regulate sources of water quality impairment

and training for State personnel. In Phase II, States are to develop plans necessary to implement biological criteria for each surface water type.

- Phase III requires full implementation and integration of biological criteria in water quality standards. This requires using biological surveys to derive biological criteria for classes of surface waters and designated uses. These criteria are then used to identify nonattainment of designated uses and make regulatory decisions.

Narrative biological criteria can be developed for all five surface water classifications with little or no data collection. Application of narrative criteria in seriously degraded waters is possible in the short term. However, because of the diversity of surface waters and the biota that inhabit these waters, significant planning, data collection, and evaluation will be needed to fully implement the program. Criteria for each type of surface water are likely to be developed at different rates. The order and rate of development will depend, in part, on the development of EPA guidance for specific types of surface water. Biological criteria technical guidance for streams will be produced during FY 1991. The tentative order for future technical guidance documents includes guidance for rivers (FY 1992), lakes (FY 1993), wetlands (FY 1994) and estuaries (FY 1995). This order and timeline for guidance does not reflect the relative importance of these surface waters, but rather indicates the relative availability of research and the anticipated difficulty of developing guidance.

## Independent Application of Biological Criteria

Biological criteria supplement, but do not replace, chemical and toxicological methods. Water chemistry methods are necessary to predict risks (particularly to human health and wildlife), and to diagnose, model, and regulate important water quality problems. Because biological criteria are able to detect different types of water quality impairments and, in particular, have different levels of sensitivity for detecting certain types of impairment compared to toxicological methods, they are not used in lieu of, or in conflict with, current regulatory efforts.

As with all criteria, certain limitations to biological criteria make independent application essential. Study design and use influences how sensitive biological criteria are for detecting community impairment. Several factors influence sensitivity: (1) State decisions about what is significantly different between reference and test communities, (2) study design, which may include community components that are not sensitive to the impact causing impairment, (3) high natural variability that makes it difficult to detect real differences, and (4) types of impacts that may be detectable sooner by other methods (e.g., chemical criteria may provide earlier indications of impairment from a bioaccumulative chemical because aquatic communities require exposure over time to incur the full effect).

Since each type of criteria (biological criteria, chemical-specific criteria, or whole-effluent toxicity evaluations) has different sensitivities and purposes, a criterion may fail to detect real impairments when used alone. As a result, these methods should be used together in an integrated water quality assessment, each providing an independent evaluation of nonattainment of a designated use. If any one type of criteria indicates impairment of the surface water, regulatory action can be taken to improve water quality. However, no one type of criteria can be used to confirm attainment of a use if another form of criteria indicates nonattainment (see Hypothesis Testing: Biological Criteria and the Scientific Method, Chapter 7). When these three methods are used together, they provide a powerful, integrated, and effective foundation for waterbody management and regulations.

## How to Use this Document

The purpose of this document is to provide EPA Regions, States and others with the conceptual framework and assistance necessary to develop and implement narrative and numeric biological criteria and to promote national consistency in application. There are two main parts of the document. Part One (Chapters 1, 2, 3, and 4) includes the essential concepts about what biological criteria are and how they are used in regulatory programs. Part Two (Chapters 5, 6, and 7) provides an overview of the process that is essential for implementing a State biological criteria program. Specific chapters include the following:

**Part I: PROGRAM ELEMENTS**

- **Chapter 2, Legal Authority,** reviews the legal basis for biological criteria under the Clean Water Act and includes possible applications under the Act and other legislation.

- **Chapter 3, Conceptual Framework,** discusses the essential program elements for biological criteria, including what they are and how they are developed and used within a regulatory program. The development of narrative biological criteria is discussed in this chapter.

- **Chapter 4, Integration,** discusses the use of biological criteria in regulatory programs.

**Part II: THE IMPLEMENTATION PROCESS**

- **Chapter 5, The Reference Condition,** provides a discussion on alternative forms of reference conditions that may be developed by a State based on circumstances and needs.

- **Chapter 6, The Biological Survey,** provides some detail on the elements of a quality biological survey.

- **Chapter 7, Hypothesis Testing: Biological Criteria and the Scientific Method,** discusses how biological surveys are used to make regulatory and diagnostic decisions.

- **Appendix A** includes commonly asked questions and their answers about biological criteria.

Two additional documents are planned in the near term to supplement this program guidance document.

1. "*Biological Criteria Technical Reference Guide*" will contain a cross reference of technical papers on available approaches and methods for developing biological criteria (see tentative table of contents in Appendix B).

2. "*Biological Criteria Development by States*" will provide a summary of different mechanisms several States have used to implement and apply biological criteria in water quality programs (see tentative outline in Appendix C).

Both documents are planned for FY 1991. As previously discussed, over the next triennium technical guidance for specific systems (e.g., streams, wetlands) will be developed to provide guidance on acceptable biological assessment procedures to further support State implementation of comprehensive programs.

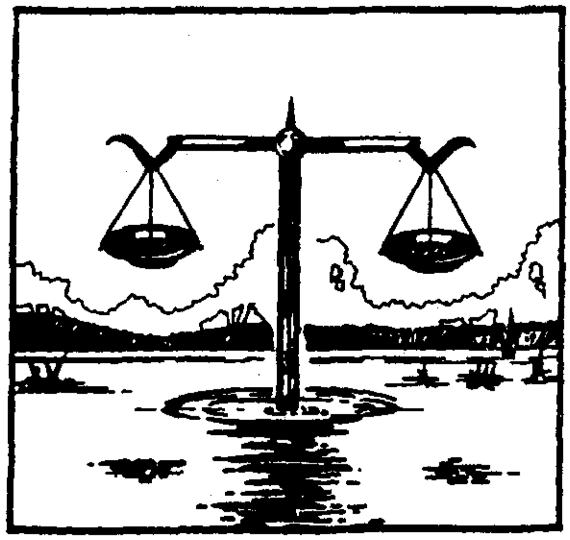
This biological criteria program guidance document supports development and implementation of biological criteria by providing guidance to States working to comply with requirements under the Clean Water Act and the Water Quality Standards Regulation. This guidance is not regulatory.

# Chapter 2

# Legal Authority

The Clean Water Act (Federal Water Pollution Control Act of 1972, Clean Water Act of 1977, and the Water Quality Act of 1987) mandates State development of criteria based on biological assessments of natural ecosystems.

The general authority for biological criteria comes from Section 101(a) of the Act which establishes as the objective of the Act the restoration and maintenance of the chemical, physical, and biological integrity of the Nation's waters. To meet this objective, water quality criteria must include criteria to protect biological integrity. Section 101(a)(2) includes the interim water quality goal for the protection and propagation of fish, shellfish, and wildlife. Propagation includes the full range of biological conditions necessary to support reproducing populations of all forms of aquatic life and other life that depend on aquatic systems. Sections 303 and 304 provide specific directives for the development of biological criteria.



*Balancing the legal authority for biological criteria.*

## Section 303

Under Section 303(c) of the Act, States are required to adopt protective water quality standards that consist of uses, criteria, and antidegradation. States are to review these standards every three years and to revise them as needed.

Section 303(c)(2)(A) requires the adoption of water quality standards that "... serve the purposes of the Act," as given in Section 101. Section 303(c)(2)(B), enacted in 1987, requires States to adopt numeric criteria for toxic pollutants for which EPA has published 304(a)(1) criteria. The section further requires that, where numeric 304(a) criteria are not available, States should adopt criteria based on biological assessment and monitoring methods, consistent with information published by EPA under 304(a)(8).

These specific directives do not serve to restrict the use of biological criteria in other settings where they may be helpful. Accordingly, this guidance document provides assistance in implementing various sections of the Act, not just 303(c)(2)(B).

## Section 304

Section 304(a) directs EPA to develop and publish water quality criteria and information on methods for measuring water quality and establishing water quality criteria for toxic pollutants on bases other than pollutant-by-pollutant, including biological monitoring and assessment methods which assess:

- the effects of pollutants on aquatic community components (". . . plankton, fish, shellfish, wildlife, plant life . . .") and community attributes (". . . biological community diversity, productivity, and stability . . ."); in any body of water and;
- factors necessary ". . . to restore and maintain the chemical, physical, and biological integrity of all navigable waters . . ." for ". . . the protection of shellfish, fish, and wildlife for classes and categories of receiving waters . . ."

## Potential Applications Under the Act

Development and use of biological criteria will help States to meet other requirements of the Act, including:

- ❑ setting planning and management priorities for waterbodies most in need of controls [Sec. 303(d)];
- ❑ determining impacts from nonpoint sources [i.e., Section 304(f) "(1) guidelines for identifying and evaluating the nature and extent of nonpoint sources of pollutants, and (2) processes, procedures, and methods to control pollution . . ."].
- ❑ biennial reports on the extent to which waters support balanced biological communities [Sec. 305(b)];
- ❑ assessment of lake trophic status and trends [Sec. 314];
- ❑ lists of waters that cannot attain designated uses without nonpoint source controls [Sec. 319];
- ❑ development of management plans and conducting monitoring in estuaries of national significance [Sec. 320];
- ❑ issuing permits for ocean discharges and monitoring ecological effects [Sec. 403(c) and 301(h)(3)];
- ❑ determination of acceptable sites for disposal of dredge and fill material [Sec. 404];

## Potential Applications Under Other Legislation

Several legislative acts require an assessment of risk to the environment (including resident aquatic communities) to determine the need for regulatory action. Biological criteria can be used in this context to support EPA assessments under:

- ❑ *Toxic Substances Control Act (TSCA) of 1976*
- ❑ *Resource Conservation and Recovery Act (RCRA),*
- ❑ *Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA),*
- ❑ *Superfund Amendments and Reauthorization Act of 1986 (SARA),*
- ❑ *Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA);*
- ❑ *National Environmental Policy Act (NEPA);*
- ❑ *Federal Lands Policy and Management Act (FLPMA).*
- ❑ *The Fish and Wildlife Conservation Act of 1980*
- ❑ *Marine Protection, Research, and Sanctuaries Act*
- ❑ *Coastal Zone Management Act*

- ***Wild and Scenic Rivers Act***

- ***Fish and Wildlife Coordination Act, as Amended in 1965***

A summary of the applicability of these Acts for assessing ecological impairments may be found in *Risk Assessment Guidance for Superfund-Environmental Evaluation Manual (Interim Final) 1989.*

Other federal and State agencies can also benefit from using biological criteria to evaluate the biological integrity of surface waters within their jurisdiction and to the effects of specific practices on surface water quality. Agencies that could benefit include:

- **Department of the Interior** (*U.S. Fish and Wildlife Service, U.S. Geological Survey, Bureau of Mines, and Bureau of Reclamation, Bureau of Indian Affairs, Bureau of Land Management, and National Park Service*).

- **Department of Commerce** (*National Oceanic and Atmospheric Administration, National Marine Fisheries Service*).

- **Department of Transportation** (*Federal Highway Administration*)

- **Department of Agriculture** (*U.S. Forest Service, Soil Conservation Service*)

- **Department of Defense,**

- **Department of Energy,**

- **Army Corps of Engineers,**

- **Tennessee Valley Authority.**

# Chapter 3

# The Conceptual Framework

Biological integrity and the determination of use impairment through assessment of ambient biological communities form the foundation for biological criteria development. The effectiveness of a biological criteria program will depend on the development of quality criteria, the refinement of use classes to support narrative criteria, and careful application of scientific principles.

## Premise for Biological Criteria

Biological criteria are based on the premise that the structure and function of an aquatic biological community within a specific habitat provide critical information about the quality of surface waters. Existing aquatic communities in pristine environments not subject to anthropogenic impact exemplify biological integrity and serve as the best possible goal for water quality. Although pristine environments are virtually non-existent (even remote waters are impacted by air pollution), minimally impacted waters exist. Measures of the structure and function of aquatic communities inhabiting unimpaired (minimally impacted) waters provide the basis for establishing a reference condition that may be compared to the condition of impacted surface waters to determine impairment.

Based on this premise, biological criteria are developed under the assumptions that: (1) surface waters subject to anthropogenic disturbance may contain impaired populations or communities of aquatic organisms—the greater the anthropogenic

*Aquatic communities assessed in unimpaired waterbodies (top) provide a reference for evaluating impairments in the same or similar waterbodies suffering from increasing anthropogenic impacts (bottom)*



18600

disturbance, the greater the likelihood and magnitude of impairment; and (2) surface waters not subject to anthropogenic disturbance generally contain unimpaired (natural) populations and communities of aquatic organisms exhibiting biological integrity.

## Biological Integrity

The expression "biological integrity" is used in the Clean Water Act to define the Nation's objectives for water quality. According to Webster's New World Dictionary (1966), integrity is, "the quality or state of being complete; unimpaired." Biological integrity has been defined 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 within a region" (Karr and Dudley 1981). For the purposes of biological criteria, these concepts are combined to develop a functional definition for evaluating biological integrity in water quality programs. Thus, biological integrity is functionally defined as:

> *the condition of the aquatic community inhabiting the unimpaired waterbodies of a specified habitat as measured by community structure and function.*

It will often be difficult to find unimpaired waters to define biological integrity and establish the reference condition. However, the structure and function of aquatic communities of high quality waters can be approximated in several ways. One is to characterize aquatic communities in the most protected waters representative of the regions where such sites exist. In areas where few or no unimpaired sites are available, characterization of least impaired systems approximates unimpaired systems. Concurrent analysis of historical records should supplement descriptions of the condition of least impaired systems. For some systems, such as lakes, evaluating paleoecological information (the record stored in sediment profiles) can provide a measure of less disturbed conditions.

Surface waters, when inhabited by aquatic communities, are exhibiting a degree of biological integrity. However, the best representation of biological integrity for a surface water should form the basis for establishing water quality goals for those waters. When tied to the development of biological criteria, the realities of limitations on biological integrity can be considered and incorporated into a progressive program to improve water quality.

## Biological Criteria

Biological criteria are narrative expressions or numerical values that describe the biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use. While biological integrity describes the ultimate goal for water quality, biological criteria are based on aquatic community structure and function for waters within a variety of designated uses. Designated aquatic life uses serve as general statements of attained or attainable uses of State waters. Once established for a designated use, biological criteria are quantifiable values used to determine whether a use is impaired, and if so, the level of impairment. This is done by specifying what aquatic community structure and function should exist in waters of a given designated use, and then comparing this condition with the condition of a site under evaluation. If the existing aquatic community measures fail to meet the criteria, the use is considered impaired.

Since biological surveys used for biological criteria are capable of detecting water quality problems (use impairments) that may not be detected by chemical or toxicity testing, violation of biological criteria is sufficient cause for States to initiate regulatory action. Corroborating chemical and toxicity testing data are not required (though they may be desirable) as supporting evidence to sustain a determination of use impairment. However, a finding that biological criteria fail to indicate use impairment does not mean the use is automatically attained. Other evidence, such as violation of physical or chemical criteria, or results from toxicity tests, can also be used to identify impairment. Alternative forms of criteria provide independent assessments of nonattainment.

As stated above, biological criteria may be narrative statements or numerical values. States can establish general narrative biological criteria early in program development without conducting biological assessments. Once established in State standards, narrative biological criteria form the legal and programmatic basis for expanding biological assessment and biosurvey programs needed to implement narrative criteria and develop numeric biological criteria. Narrative biological criteria should become part of State regulations and standards.

## *Narrative Criteria*

Narrative biological criteria are general statements of attainable or attained conditions of biological integrity and water quality for a given use designation. Although similar to the "free from" chemical water quality criteria, narrative biological criteria establish a positive statement about what should occur within a water body. Narrative criteria can take a number of forms but they must contain several attributes to support the goals of the Clean Water Act to provide for the protection and propagation of fish, shellfish, and wildlife. Thus, narrative criteria should include specific language about aquatic community characteristics that (1) must exist in a waterbody to meet a particular designated aquatic life use, and (2) are quantifiable. They must be written to protect the use. Supporting statements for the criteria should promote water quality to protect the most natural community possible for the designated use. Mechanisms should be established in the standard to address potentially conflicting multiple uses. Narratives should be written to protect the most sensitive use and support antidegradation.

Several States currently use narrative criteria. In Maine, for example, narrative criteria were established for four classes of water quality for streams and rivers (see Table 4). The classifications were based on the range of goals in the Act from "no discharge" to "protection and propagation of fish, shellfish, and wildlife" (Courtemanch and Davies 1987). Maine separated its "high quality water" into two categories, one that reflects the highest goal of the Act (no discharge, Class AA) and one that reflects high integrity but is minimally impacted by human activity (Class A). The statement "The aquatic life . . . shall be as naturally occurs" is a narrative biological criterion for both Class AA and A waters. Waters in Class B meet the use when the life stages of all indigenous aquatic species are supported and no detrimental changes occur in community composition (Maine DEP 1986). These criteria directly support refined designated aquatic life uses (see Section D, Refining Aquatic Life Use Classifications).

These narrative criteria are effective only if, as Maine has done, simple phrases such as "as naturally occurs" and "nondetrimental" are clearly operationally defined. Rules for sampling procedures and data analysis and interpretation should become part of the regulation or supporting documentation. Maine was able to develop these criteria and their supporting statements using avail-

**Table 4.—Aquatic Life Classification Scheme for Maine's Rivers and Streams.**

| RIVERS AND STREAMS | MANAGEMENT PERSPECTIVE | LEVEL OF BIOLOGICAL INTEGRITY |
|---|---|---|
| Class AA | High quality water for preservation of recreational and ecological interests. No discharges of any kind permitted. No impoundment permitted. | Aquatic life shall be as naturally occurs. |
| Class A | High quality water with limited human interference. Discharges restricted to noncontact process water or highly treated wastewater of quality equal to or better than the receiving water. Impoundment permitted. | Aquatic life shall be as naturally occurs |
| Class B | Good quality water. Discharges of well treated effluents with ample dilution permitted. | Ambient water quality sufficient to support life stages of all indigenous aquatic species. Only nondetrimental changes in community composition may occur. |
| Class C | Lowest quality water. Requirements consistent with interim goals of the federal Water Quality Law (fishable and swimmable) | Ambient water quality sufficient to support the life stages of all indigenous fish species. Changes in species composition may occur but structure and function of the aquatic community must be maintained |



18602

able data from water quality programs. To implement the criteria, aquatic life inhabiting unimpaired waters must be measured to quantify the criteria statement.

Narrative criteria can take more specific forms than illustrated in the Maine example. Narrative criteria may include specific classes and species of organisms that will occur in waters for a given designated use. To develop these narratives, field evaluations of reference conditions are necessary to identify biological community attributes that differ significantly between designated uses. For example in the Arkansas use class <u>Typical Gulf Coastal Ecoregion</u> (i.e., South Central Plains) the narrative criterion reads:

> *"Streams supporting diverse communities of indigenous or adapted species of fish and other forms of aquatic life. Fish communities are characterized by a limited proportion of sensitive species; sunfishes are distinctly dominant, followed by darters and minnows. The community may be generally characterized by the following fishes: Key Species—Redfin shiner, Spotted sucker, Yellow bullhead, Flier, Slough darter, Grass pickerel; Indicator Species—Pirate perch, Warmouth, Spotted sunfish, Dusky darter, Creek chubsucker, Banded pygmy sunfish (Arkansas DPCE 1988).*

In Connecticut, current designated uses are supported by narratives in the standard. For example, under Surface Water Classifications, Inland Surface Waters Class AA, the Designated Use is: "Existing or proposed drinking water supply; fish and wildlife habitat; recreational use; agricultural, industrial supply, and other purposes (recreation uses may be restricted)."

The supporting narratives include:

> *Benthic invertebrates which inhabit lotic waters: A wide variety of macroinvertebrate taxa should normally be present and all functional groups should normally be well represented . . . Water quality shall be sufficient to sustain a diverse macroinvertebrate community of indigenous species. Taxa within the Orders Plecoptera (stoneflies), Ephemeroptera (mayflies), Coleoptera (beetles), Tricoptera (caddisflies) should be well represented (Connecticut DEP 1987).*

For these narratives to be effective in a biological criteria program expressions such as "a wide variety" and "functional groups should normally be well represented" require quantifiable definitions that become part of the standard or supporting documentation. Many States may find such narratives in their standards already. If so, States should evaluate current language to determine if it meets the requirements of quantifiable narrative criteria that support refined aquatic life uses.

Narrative biological criteria are similar to the traditional narrative "free froms" by providing the legal basis for standards applications. A sixth "free from" could be incorporated into standards to help support narrative biological criteria such as "free from activities that would impair the aquatic community as it naturally occurs." Narrative biological criteria can be used immediately to address obvious existing problems.

## Numeric Criteria

Numerical indices that serve as biological criteria should describe expected attainable community attributes for different designated uses. It is important to note that full implementation of narrative criteria will require similar data as that needed for developing numeric criteria. At this time, States may or may not choose to establish numeric criteria but may find it an effective tool for regulatory use.

To derive a numeric criterion, an aquatic community's structure and function is measured at reference sites and set as a reference condition. Examples of relative measures include similarity indices, coefficients of community loss, and comparisons of lists of dominant taxa. Measures of existing community structure such as species richness, presence or absence of indicator taxa, and distribution of trophic feeding groups are useful for establishing the normal range of community components to be expected in unimpaired systems. For example, Ohio uses criteria for the warmwater habitat use class based on multiple measures in different reference sites within the same ecoregion. Criteria are set as the 25th percentile of all biological index scores recorded at established reference sites within the ecoregion. Exceptional warmwater habitat index criteria are set at the 75th percentile (Ohio EPA 1988a). Applications such as this require an extensive data base and multiple reference sites for each criteria value.

To develop numeric biological criteria, careful assessments of biota in reference sites must be conducted (Hughes et al. 1986). There are numerous ways to assess community structure and function in surface waters. No single index or measure is universally recognized as free from bias. It is important to evaluate the strengths and weaknesses of different assessment approaches. A multimetric approach that incorporates information on species richness, trophic composition, abundance or biomass, and organism condition is recommended. Evaluations that measure multiple components of communities are also recommended because they tend to be more reliable (e.g., measures of fish and macroinvertebrates combined will provide more information than measures of fish communities alone). The weaknesses of one measure or index can often be compensated by combining it with the strengths of other community measurements.

The particular indices used to develop numeric criteria depend on the type of surface waters (streams, rivers, lakes, Great Lakes, estuaries, wetlands, and nearshore marine) to which they must be applied. In general, community-level indices such as the Index of Biotic Integrity developed for midwestern streams (Karr et al. 1986) are more easily interpreted and less variable than fluctuating numbers such as population size. Future EPA technical guidance documents will include evaluations of the effectiveness of different biological survey and assessment approaches for measuring the biological integrity of surface water types and provide guidance on acceptable approaches for biological criteria development.

# Refining Aquatic Life Use Classifications

State standards consist of (1) designated aquatic life uses, (2) criteria sufficient to protect the designated and existing use, and (3) an antidegradation clause. Biological criteria support designated aquatic life use classifications for application in State standards. Each State develops its own designated use classification system based on the generic uses cited in the Act (e.g., protection and propagation of fish, shellfish, and wildlife). Designated uses are intentionally general. However, States may develop subcategories within use designations to refine and clarify the use class. Clarification of the use class is particularly helpful when a variety of surface waters with distinct characteristics fit within the same use class, or do not fit well into any category. Determination of nonattainment in these waters may be difficult and open to alternative interpretations. If a determination is in dispute, regulatory actions will be difficult to accomplish. Emphasizing aquatic community structure within the designated use focuses the evaluation of attainment/nonattainment on the resource of concern under the Act.

Flexibility inherent in the State process for designating uses allows the development of subcategories of uses within the Act's general categories. For example, subcategories of aquatic life uses may be on the basis of attainable habitat (e.g., cold versus warmwater habitat); innate differences in community structure and function, (e.g., high versus low species richness or productivity); or fundamental differences in important community components (e.g., warmwater fish communities dominated by bass versus catfish). Special uses may also be designated to protect particularly unique, sensitive, or valuable aquatic species, communities, or habitats.

Refinement of use classes can be accomplished within current State use classification structures. Data collected from biosurveys as part of a developing biocriteria program may reveal unique and consistent differences among aquatic communities inhabiting different waters with the same designated use. Measurable biological attributes could then be used to separate one class into two or more classes. The result is a refined aquatic life use. For example, in Arkansas the beneficial use Fisheries "provides for the protection and propagation of fish, shellfish, and other forms of aquatic life" (Arkansas DPCE 1988). This use is subdivided into Trout, Lakes and Reservoirs, and Streams. Recognizing that stream characteristics across regions of the State differed ecologically, the State further subdivided the stream designated uses into eight additional uses based on regional characteristics (e.g. Springwater-influenced Gulf Coastal Ecoregion Ouachita Mountains Ecoregion). Within this classification system, it was relatively straightforward for



18604

Arkansas to establish detailed narrative biological criteria that list aquatic community components expected in each ecoregion (see Narrative Criteria section). These narrative criteria can then be used to establish whether the use is impaired.

States can refine very general designated uses such as high, medium, and low quality to specific categories that include measurable ecological characteristics. In Maine, for example, Class AA waters are defined as "the highest classification and shall be applied to waters which are outstanding natural resources and which should be preserved because of their ecological, social, scenic, or recreational importance." The designated use includes 'Class AA waters shall be of such quality that they are suitable . . . as habitat for fish and other aquatic life. The habitat shall be characterized as free flowing and natural." This use supports development of narrative criteria based on biological characteristics of aquatic communities (Maine DEP 1986; see the Narrative Criteria section).

Biological criteria that include lists of dominant or typical species expected to live in the surface water are particularly effective. Descriptions of impaired conditions are more difficult to interpret. However, biological criteria may contain statements concerning which species dominate disturbed sites, as well as those species expected at minimally impacted sites.

Most States collect biological data in current programs. Refining aquatic life use classifications and incorporating biological criteria into standards will enable States to evaluate these data more effectively.

# Developing and Implementing Biological Criteria

Biological criteria development and implementation in standards require an understanding of the selection and evaluation of reference sites, measurement of aquatic community structure and function, and hypothesis testing under the scientific method. The developmental process is important for State water quality managers and their staff to understand to promote effective planning for resource and staff needs. This major program element deserves careful consideration and has been separated out in Part II by chapter for each developmental step as noted below. Additional guidance will be provided in future technical guidance documents.

The developmental process is illustrated in Figure 3. The first step is establishing narrative criteria in standards. However, to support these narratives, standardized protocols need to be developed to quanitify the narratives for criteria implementation. They should include data collection procedures, selection of reference sites, quality assurance and quality control procedures, hypothesis testing, and statistical protocols. Pilot studies should be conducted using these standard protocols to ensure they meet the needs of the program, test the hypotheses, and provide effective measures of the biological integrity of surface waters in the State.

**Figure 3.—Process for the Development and Implementation of Biological Criteria**

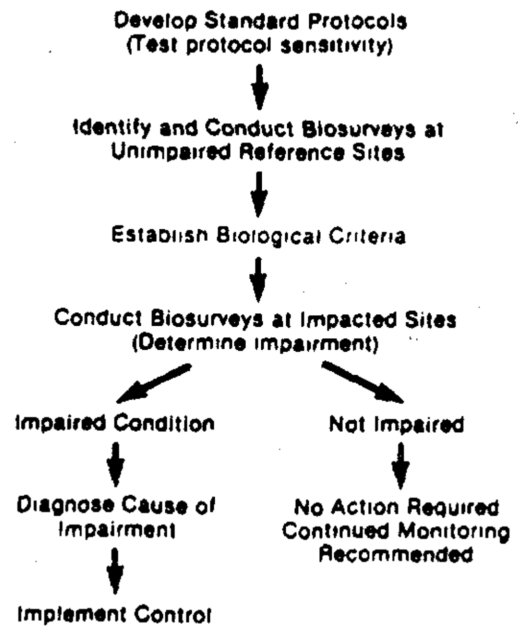



Fig. 3: Implementation of biological criteria requires the initial selection of reference sites and characterization of resident aquatic communities inhabiting those sites to establish the reference condition and biological criteria. After criteria development, impacted sites are evaluated using the same biosurvey procedures to assess resident biota. If impairment is found, diagnosis of cause will lead to the implementation of a control. Continued monitoring should accompany control implementation to determine the effectiveness of intervention. Monitoring is also recommended where no impairment is found to ensure that the surface water maintains or improves in quality.

The next step is establishing the reference condition for the surface water being tested. This reference may be site specific or regional but must establish the unimpaired baseline for comparison (see Chapter 5, The Reference Condition). Once reference sites are selected, the biological integrity of the site must be evaluated using carefully chosen biological surveys. A quality biological survey will include multiple community components and may be measured using a variety of metrics (see Chapter 6, The Biological Survey). Establishing the reference condition and conducting biological surveys at the reference locations provide the necessary information for establishing the biological criteria.

To apply biological criteria, impacted surface waters with comparable habitat characteristics are evaluated using the same procedures as those used to establish the criteria. The biological survey must support standardized sampling methods and statistical protocols that are sensitive enough to identify biologically relevant differences between established criteria and the community under evaluation. Resulting data are compared through hypothesis testing to determine impairment (see Chapter 7, Hypothesis Testing).

When water quality impairments are detected using biological criteria, they can only be applied in a regulatory setting if the cause for impairment can be identified. Diagnosis is iterative and investigative (see Chapter 7, Diagnosis). States must then determine appropriate actions to implement controls. Monitoring should remain a part of the biological criteria program whether impairments are found or not. If an impairment exists, monitoring provides a mechanism to determine if the control effort (intervention) is resulting in improved water quality. If there is no impairment, monitoring ensures the water quality is maintained and documents any improvements. When improvements in water quality are detected through monitoring programs two actions are recommended. When reference condition waters improve, biological criteria values should be recalculated to reflect this higher level of integrity. When impaired surface waters improve, states should reclassify those waters to reflect a refined designated use with a higher level of biological integrity. This provides a mechanism for progressive water quality improvement.

# Chapter 4

# *Integrating Biological Criteria Into Surface Water Management*

Integrating biological criteria into existing water quality programs will help to assess use attainment/nonattainment, improve problem discovery in specific waterbodies, and characterize overall water resource condition within a region. Ideally, biological criteria function in an iterative manner. New biosurvey information can be used to refine use classes. Refined use classes will help support criteria development and improve the value of data collected in biosurveys.

*To integrate biological criteria into water quality programs, states must carefully determine where and how data are collected to assess the biological integrity of surface waters.*

## Implementing Biological Criteria

As biological survey data are collected, these data will increasingly support current use of biomonitoring data to identify water quality problems, assess their severity, and set planning and management priorities for remediation. Monitoring data and biological criteria should be used at the outset to help make regulatory decisions, develop appropriate controls, and evaluate the effectiveness of controls once they are implemented.

The value of incorporating biological survey information in regulatory programs is illustrated by evaluations conducted by North Carolina. In response to amendments of the Federal Water Pollution Control Act requiring secondary effluent limits for all wastewater treatment plants, North Carolina became embroiled in a debate over whether meeting secondary effluent limits (at considerable cost) would result in better water quality. North Carolina chose to test the effectiveness of additional treatment by conducting seven chemical and biological surveys before and after facility upgrades (North

Carolina DNRCD 1984). Study results indicated that moderate to substantial in-stream improvements were observed at six of seven facilities. Biological surveys were used as an efficient, cost-effective monitoring tool for assessing in-stream improvements after facility modification. North Carolina has also conducted comparative studies of benthic macroinvertebrate surveys and chemical-specific and whole-effluent evaluations to assess sensitivities of these measures for detecting impairments (Eagleson et al. 1990).

Narrative biological criteria provide a scientific framework for evaluating biosurvey, bioassessment, and biomonitoring data collected in most States. Initial application of narrative biological criteria may require only an evaluation of current work. States can use available data to define variables for choosing reference sites, selecting appropriate biological surveys, and assessing the response of local biota to a variety of impacts. States should also consider the decision criteria that will be used for determining appropriate State action when impairment is found.

Recent efforts by several States to develop biological criteria for freshwater streams provide excellent examples for how biological criteria can be integrated into water quality programs. Some of this work is described in the *National Workshop on In-stream Biological Monitoring and Criteria* proceedings which recommended that "the concept of biological sampling should be integrated into the full spectrum of State and Federal surface water programs" (U.S. EPA 1987b). States are actively developing biological assessment and criteria programs; several have programs in place.

# Biological Criteria in State Programs

Biological criteria are used within water programs to refine use designations, establish criteria for determining use attainment/nonattainment, evaluate effectiveness of current water programs, and detect and characterize previously unknown impairments. Twenty States are currently using some form of standardized ambient biological assessments to determine the status of biota within State waters. Levels of effort vary from bioassessment studies to fully developed biological criteria programs.

Fifteen States are developing aspects of biological assessments that will support future development of biological criteria. Colorado, Illinois, Iowa, Kentucky, Massachusetts, Tennessee, and Virginia conduct biological monitoring to evaluate biological conditions, but are not developing biological criteria. Kansas is considering using a community metric for water resource assessment. Arizona is planning to refine ecoregions for the State. Delaware, Minnesota, Texas, and Wisconsin are developing sampling and evaluation methods to apply to future biological criteria programs. New York is proposing to use biological criteria for site-specific evaluations of water quality impairment. Nebraska and Vermont use informal biological criteria to support existing aquatic life narratives in their water quality standards and other regulations. Vermont recently passed a law requiring that biological criteria be used to regulate through permitting the indirect discharge of sanitary effluents.

Florida incorporated a specific biological criterion into State standards for invertebrate species diversity. Species diversity within a waterbody, as measured by a Shannon diversity index, may not fall below 75 percent of reference values. This criterion has been used in enforcement cases to obtain injunctions and monetary settlements. Florida's approach is very specific and limits alternative applications.

Four States—Arkansas, North Carolina, Maine, and Ohio—are currently using biological criteria to define aquatic life use classifications and enforce water quality standards. These states have made biological criteria an integral part of comprehensive water quality programs.

- Arkansas rewrote its aquatic life use classifications for each of the State's ecoregions. This has allowed many cities to design wastewater treatment plants to meet realistic attainable dissolved oxygen conditions as determined by the new criteria.

- North Carolina developed biological criteria to assess impairment to aquatic life uses written as narratives in the State water quality standards. Biological data and criteria are used extensively to identify waters of special concern or those with exceptional water quality. In addition to the High Quality Waters (HQW) and Outstanding Resource Waters (ORW) designations, Nutrient Sensitive Waters (NSW) at risk for eutrophication are assessed using biological criteria. Although specific biological measures are not in the regulations, strengthened use of biological monitoring data to assess water quality is being proposed for incorporation in North Carolina's water quality standards.

■ **Maine** has enacted a revised Water Quality Classification Law specifically designed to facilitate the use of biological assessments. Each of four water classes contains descriptive aquatic life conditions necessary to attain that class. Based on a statewide database of macroinvertebrate samples collected above and below outfalls, Maine is now developing a set of dichotomous keys that serve as the biological criteria. Maine's program is not expected to have a significant role in permitting, but will be used to assess the degree of protection afforded by effluent limitations.

■ **Ohio** has instituted the most extensive use of biological criteria for defining use classifications and assessing water quality. Biological criteria were developed for Ohio rivers and streams using an ecoregional reference site approach. Within each of the State's five ecoregions, criteria for three biological indices (two for fish communities and one for macroinvertebrates) were derived. Ohio successfully uses biological criteria to demonstrate attainment of aquatic life uses and discover previously unknown or unidentified environmental degradation (e.g., twice as many impaired waters were discovered using biological criteria and water chemistry together than were found using chemistry alone). The upgraded use designations based on biological criteria were upheld in Ohio courts and the Ohio EPA successfully proposed their biological criteria for inclusion in the State water quality standards regulations.

States and EPA have learned a great deal about the effectiveness of integrated biological assessments through the development of biological criteria for freshwater streams. This information is particularly valuable in providing guidance on developing biological criteria for other surface water types. As previously discussed, EPA plans to produce supporting technical guidance for biological criteria development in streams and other surface waters. Production of these guidance documents will be contingent on technical progress made on each surface water type by researchers in EPA, States and the academic community.

EPA will also be developing outreach workshops to provide technical assistance to Regions and States working toward the implementation of biological criteria programs in State water quality management programs. In the interim, States should use the technical guidance currently available in the *Technical Support Manual(s): Waterbody Surveys and Assessments for Conducting Use Attainability Analysis* (U.S. EPA 1983b, 1984a,b).

During the next triennium, State effort will be focused on developing narrative biological criteria. Full implementation and integration of biological criteria will require several years. Using available guidance, States can complement the adoption of narrative criteria by developing implementation plans that include:

1. Defining program objectives, developing research protocols, and setting priorities;
2. Determining the process for establishing reference conditions, which includes developing a process to evaluate habitat characteristics;
3. Establishing biological survey protocols that include justifications for surface water classifications and selected aquatic community components to be evaluated; and
4. Developing a formal document describing the research design, quality assurance and quality control protocols, and required training for staff.

Whether a State begins with narrative biological criteria or moves to fully implement numeric criteria, the shift of the water quality program focus from source control to resource management represents a natural progression in the evolution from the technology-based to water quality-based approaches in water quality management. The addition of a biological perspective allows water quality programs to more directly address the objectives of the Clean Water Act and to place their efforts in a context that is more meaningful to the public.

# Future Directions

Biological criteria now focus on resident aquatic communities in surface waters. They have the potential to expand in scope toward greater ecological integration. Ecological criteria may encompass the ambient aquatic communities in surface waters, wildlife species that use the same aquatic resources, and the aquatic community inhabiting the gravel and sediments underlying the surface waters and adjacent land (hyporheic zone); specific criteria may apply to physical habitat. These areas may represent only a few possible options for biological criteria in the future.

Many wildlife species depend on aquatic resources. If aquatic population levels decrease or if the distribution of species changes, food sources may be sufficiently altered to cause problems for wildlife species using aquatic resources. Habitat degradation that impairs aquatic species will often impact important wildlife habitat as well. These kinds of impairments are likely to be detected using biological criteria as currently formulated. In some cases, however, uptake of contaminants by resident aquatic organisms may not result in altered structure and function of the aquatic community. These impacts may go undetected by biological criteria, but could result in wildlife impairments because of bioaccumulation. Future expansion of biological criteria to include wildlife species that depend on aquatic resources could provide a more integrative ecosystem approach.

Rivers may have a subsurface flood plain extending as far as two kilometers from the river channel. Preliminary mass transport calculations made in the Flathead River basin in Montana indicate that nutrients discharged from this subsurface flood plain may be crucial to biotic productivity in the river channel (Stanford and Ward 1988). This is an unexplored dimension in the ecology of gravel river beds and potentially in other surface waters.

As discussed in Chapter 1, physical integrity is a necessary condition for biological integrity. Establishing the reference condition for biological criteria requires evaluation of habitat. The rapid bioassessment protocol provides a good example of the importance of habitat for interpreting biological assessments (Plafkin et al. 1989). However, it may be useful to more fully integrate habitat characteristics into the regulatory process by establishing criteria based on the necessary physical structure of habitats to support ecological integrity.

# Part II

# *The Implementation Process*

18611

The implementation of biological criteria requires: (1) selection of unimpaired (minimal impact) surface waters to use as the reference condition for each designated use, (2) measurement of the structure and function of aquatic communities in reference surface waters to establish biological criteria, and (3) establishment of a protocol to compare the biological criteria to biota in impacted waters to determine whether impairment has occurred. These elements serve as an interactive network that is particularly important during early development of biological criteria where rapid accumulation of information is effective for refining both designated uses and developing biological criteria values. The following chapters describe these three essential elements.

# Chapter 5

# *The Reference Condition*

A key step in developing values for supporting narrative and creating numeric biological criteria is to establish reference conditions; it is an essential feature of environmental impact evaluations (Green 1979). Reference conditions are critical for environmental assessments because standard experimental controls are rarely available. For most surface waters, baseline data were not collected prior to an impact, thus impairment must be inferred from differences between the impact site and established references. Reference conditions describe the characteristics of waterbody segments least impaired by human activities and are used to define attainable biological or habitat conditions.

*Reference conditions should be established by measuring resident biota in unimpaired surface waters.*

Wide variability among natural surface waters across the country resulting from climatic, landform, and other geographic differences prevents the development of nationwide reference conditions. Most States are also too heterogeneous for single reference conditions. Thus, each State, and when appropriate, groups of States, will be responsible for selecting and evaluating reference waters within the State to establish biological criteria for a given surface water type or category of designated use. At least seven methods for estimating attainable conditions for streams have been identified (Hughes et al. 1986). Many of these can apply to other surface waters. References may be established by defining models of attainable conditions based on historical data or unimpaired habitat (e.g., streams in old growth forest). The reference condition established as before-after comparisons or concurrent measures of the reference water and impact sites can be based on empirical data (Hall et al. 1989).

Currently, two principal approaches are used for establishing the reference condition. A State may opt to (1) identify site-specific reference sites for each evaluation of impact or (2) select ecologically similar regional reference sites for comparison with impacted sites within the same region. Both approaches depend on evaluations of habitats to ensure that waters with similar habitats are compared. The designation of discrete habitat types is more fully developed for streams and rivers. Development of habitat types for lakes, wetlands, and estuaries is ongoing.

# Site-Specific Reference Condition

A site-specific reference condition, frequently used to evaluate the impacts from a point discharge, is best for surface waters with a strong directional flow such as in streams and rivers (the upstream-downstream approach). However, it can also be used for other surface waters where gradients in contaminant concentration occur based on proximity to a source (the near field-far field approach). Establishment of a site-specific reference condition requires the availability of comparable habitat within the same waterbody in both the reference location and the impacted area.

A site-specific reference condition is difficult to establish if (1) diffuse nonpoint source pollution contaminates most of the water body; (2) modifications to the channel, shoreline, or bottom substrate are extensive; (3) point sources occur at multiple locations on the waterbody; or (4) habitat characteristics differ significantly between possible reference locations and the impact site (Hughes et al. 1986; Plafkin et al. 1989). In these cases, site-specific reference conditions could result in underestimates of impairment. Despite limitations, the use of site-specific reference conditions is often the method of choice for point source discharges and certain waterbodies, particularly when the relative impairments from different local impacts need to be determined.

## *The Upstream-Downstream Reference Condition*

The upstream-downstream reference condition is best applied to streams and rivers where the habitat characteristics of the waterbody above the point of discharge are similar to the habitat characteristics of the stream below the point of discharge. One standard procedure is to characterize the biotic condition just above the discharge point (accounting for possible upstream circulation) to establish the reference condition. The condition below the discharge is also measured at several sites. If significant differences are found between these measures, impairment of the biota from the discharge is indicated. Since measurements of resident biota taken in any two sites are expected to differ because of natural variation, more than one biological assessment for both upstream and downstream sites is often needed to be confident in conclusions drawn from these data (Green, 1979). However, as more data are collected by a State, and particularly if regional characteristics of the waterbodies are incorporated, the basis for determining impairment from site-specific upstream-downstream assessments may require fewer individual samples. The same measures made below the "recovery zone" downstream from the discharge will help define where recovery occurs.

The upstream-downstream reference condition should be used with discretion since the reference condition may be impaired from impacts upstream from the point source of interest. In these cases it is important to discriminate between individual point source impact versus overall impairment of the system. When overall impairment occurs, the resident biota may be sufficiently impaired to make it impossible to detect the effect of the target point source discharger.

The approach can be cost effective when one biological assessment of the upstream reference condition adequately reflects the attainable condition of the impacted site. However, routine comparisons may require assessments of several upstream sites to adequately describe the natural variability of reference biota. Even so, measuring a series of site-specific references will likely continue to be the method of choice for certain point source discharges, especially where the relative impairments from different local impacts need to be determined.

## *The Near Field-Far Field Reference Condition*

The near field-far field reference condition is effective for establishing a reference condition in surface waters other than rivers and streams and is particularly applicable for unique waterbodies (e.g., estuaries such as Puget Sound may not have comparable estuaries for comparison). To apply this method, two variables are measured (1) habitat characteristics, and (2) gradient of impairment. For reference waters to be identified within the same waterbody, sufficient size is necessary to separate the reference from the impact area so that a gradient of impact exists. At the same time, habitat characteristics must be comparable.



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Although not fully developed, this approach may provide an effective way to establish biological criteria for estuaries, large lakes, or wetlands. For example, estuarine habitats could be defined and possible reference waters identified using physical and chemical variables like those selected by the Chesapeake Bay Program (U.S. EPA 1987a, e.g., substrate type, salinity, pH) to establish comparable subhabitats in an estuary. To determine those areas least impaired, a "mussel watch" program like that used in Narragansett Bay (i.e., captive mussels are used as indicators of contamination, (Phelps 1988)) could establish impairment gradients. These two measures, when combined, could form the basis for selecting specific habitat types in areas of least impairment to establish the reference condition.

# Regional Reference Conditions

Some of the limitations of site-specific reference conditions can be overcome by using regional reference conditions that are based on the assumption that surface waters integrate the character of the land they drain. Waterbodies within the same watershed in the same region should be more similar to each other than to those within watersheds in different regions. Based on these assumptions, a distribution of aquatic regions can be developed based on ecological features that directly or indirectly relate to water quality and quantity, such as soil type, vegetation (land cover), land-surface form, climate, and land use. Maps that incorporate several of these features will provide a general purpose broad scale ecoregional framework (Gallant et al. 1989).

Regions of ecological similarity are based on hydrologic, climatic, geologic, or other relevant geographic variables that influence the nature of biota in surface waters. To establish a regional reference condition, surface waters of similar habitat type are identified in definable ecological regions. The biological integrity of these reference waters is determined to establish the reference condition and develop biological criteria. These criteria are then used to assess impacted surface waters in the same watershed or region. There are two forms of regional reference conditions: (1) paired watersheds and (2) ecoregions.

## *Paired Watershed Reference Conditions*

Paired watershed reference conditions are established to evaluate impaired waterbodies, often impacted by multiple sources. When the majority of a waterbody is impaired, the upstream-downstream or near field-far field reference condition does not provide an adequate representation of the unimpaired condition of aquatic communities for the waterbody. Paired watershed reference conditions are established by identifying unimpaired surface waters within the same or very similar local watershed that is of comparable type and habitat. Variables to consider when selecting the watershed reference condition include absence of human disturbance, waterbody size and other physical characteristics, surrounding vegetation, and others as described in the "Regional Reference Site Selection" feature.

This method has been successfully applied (e.g., Hughes 1985) and is an approach used in Rapid Bioassessment Protocols (Plafkin et al. 1989). State use of this approach results in good reference conditions that can be used immediately in current programs. This approach has the added benefit of promoting the development of a database on high quality waters in the State that could form the foundation for establishing larger regional references (e.g., ecoregions.)

## *Ecoregional Reference Conditions*

Reference conditions can also be developed on a larger scale. For these references, waterbodies of similar type are identified in regions of ecological similarity. To establish a regional reference condition, a set of surface waters of similar habitat type are identified in each ecological region. These sites must represent similar habitat type and be representative of the region. As with other reference conditions, the biological integrity of selected reference waters is determined to establish the reference. Biological criteria can then be developed and used to assess impacted surface waters in the same region. Before reference conditions may be established, regions of ecological similarity must be defined.

## Regional Reference Site Selection

*To determine specific regional reference sites for streams, candidate watersheds are selected from the appropriate maps and evaluated to determine if they are typical for the region. An evaluation of level of human disturbance is made and a number of relatively undisturbed reference sites are selected from the candidate sites. Generally, watersheds are chosen as regional reference sites when they fall entirely within typical areas of the region. Candidate sites are then selected by aerial and ground surveys. Identification of candidate sites is based on: (1) absence of human disturbance, (2) stream size, (3) type of stream channel, (4) location within a natural or political refuge and (5) historical records of resident biota and possible migration barriers.*

*Final selection of reference sites depends on a determination of minimal disturbance derived from habitat evaluation made during site visits. For example, indicators of good quality streams in forested ecoregions include: (1) extensive, old, natural riparian vegetation; (2) relatively high heterogeneity in channel width and depth; (3) abundant large woody debris, coarse bottom substrate or extensive aquatic or overhanging vegetation, (4) relatively high or constant discharge, (5) relatively clear waters with natural color and odor, (6) abundant diatom, insect, and fish assemblages; and (7) the presence of piscivorous birds and mammals.*

One frequently used method is described by Omernik (1987) who combined maps of land-surface form, soil, potential natural vegetation, and land use within the conterminous United States to generate a map of aquatic ecoregions for the country. He also developed more detailed regional maps. The ecoregions defined by Omernik have been evaluated for streams and small rivers in Arkansas (Rohm et al. 1987), Ohio (Larsen et al. 1986; Whittier et al. 1987), Oregon (Whittier et al. 1988), Colorado (Gallant et al. 1989), and Wisconsin (Lyons 1989) and for lakes in Minnesota (Heiskary et al. 1987). State ecoregion maps were developed for Colorado (Gallant et al. 1989) and Oregon (Clarke et al. mss). Maps for the national ecoregions and six multi-state maps of more detailed ecoregions are available from the U.S. EPA Environmental Research Laboratory, Corvallis, Oregon.

Ecoregions such as those defined by Omernik (1987) provide only a first step in establishing regional reference sites for development of the reference condition. Field site evaluation is required to account for the inherent variability within each ecoregion. A general method for selecting reference sites for streams has been described (Hughes et al. 1986). These are the same variables used for comparable watershed reference site selection. Regional and on-site evaluations of biological factors help determine specific sites that best represent typical but unimpaired surface water habitats within the region. Details on this approach for streams is described in the "Regional Reference Site Selection" feature. To date, the regional approach has been tested on streams, rivers, and lakes. The method appears applicable for assessing other inland ecosystems. To apply this approach to wetlands and estuaries will require additional evaluation based on the relevant ecological features of these ecosystems (e.g. Brooks and Hughes, 1988).

Ideally, ecoregional reference sites should be as little disturbed as possible, yet represent waterbodies for which they are to serve as reference waters. These sites may serve as references for a large number of similar waterbodies (e.g., several reference streams may be used to define the reference condition for numerous physically separate streams if the reference streams contain the same range of stream morphology, substrate, and flow of the other streams within the same ecological region).

An important benefit of a regional reference system is the establishment of a baseline condition for the least impacted surface waters within the dominant land use pattern of the region. In many areas a return to pristine, or presettlement, conditions is impossible, and goals for waterbodies in extensively developed regions could reflect this. Regional reference sites based on the least impacted sites within a region will help water quality programs restore and protect the environment in a way that is ecologically feasible.

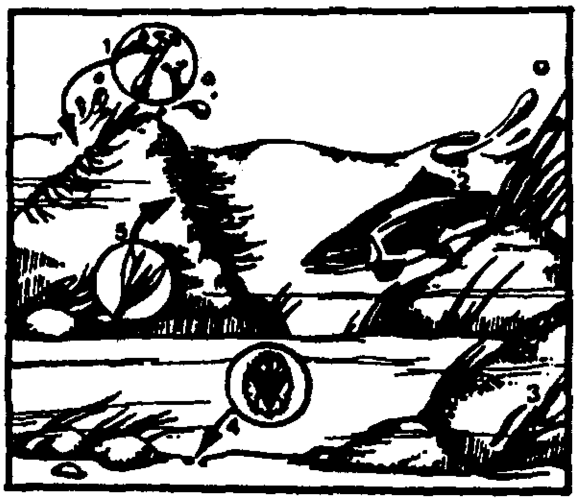
This approach must be used with caution for two reasons. First, in many urban, industrial, or heavily developed agricultural regions, even the least impacted sites are seriously degraded. Basing standards or criteria on such sites will set standards too low if these high levels of environmental degradation are considered acceptable or adequate. In such degraded regions, alternative sources for the regional reference may be needed (e.g., measures taken from the same region in a less developed neighboring State or historical records from the region before serious impact occurred). Second, in some regions the minimally-impacted sites are not typical of most sites in the region and may have remained unimpaired precisely because they are unique. These two considerations emphasize the need to select reference sites very carefully, based on solid quantitative data interpreted by professionals familiar with the biota of the region.

Each State, or groups of States, can select a series of regional reference sites that represent the attainable conditions for each region. Once biological criteria are established using this approach, the cost for evaluating local impairments is often lower than a series of measures of site-specific reference sites. Using paired watershed reference conditions immediately in regulatory programs will provide the added benefit of building a database for the development of regions of ecological similarity.

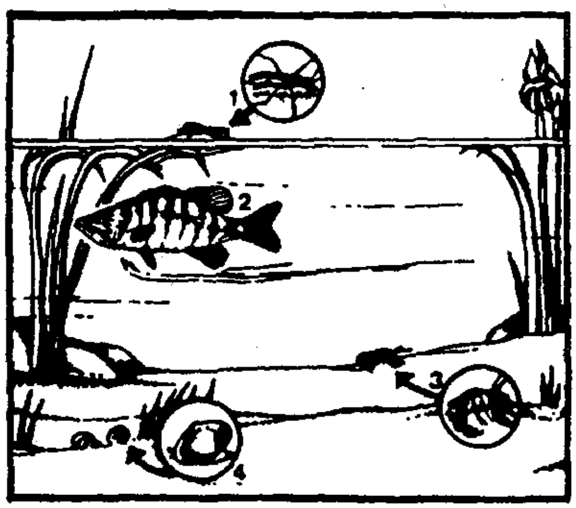
# Chapter 6

# *The Biological Survey*

A critical element of biological criteria is the characterization of biological communities inhabiting surface waters. Use of biological data is not new; biological information has been used to assess impacts from pollution since the 1890s (Forbes 1928), and most States currently incorporate biological information in their decisions about the quality of surface waters. However, biological information can be obtained through a variety of methods, some of which are more effective than others for characterizing resident aquatic biota. Biological criteria are developed using biological surveys; these provide the only direct method for measuring the structure and function of an aquatic community.



*Different subhabitat within the same surface water will contain unique aquatic community components. In fast-flowing stream segments species such as (1) black fly larva; (2) brook trout; (3) water penny; (4) crane fly larva; and (5) water moss occur.*



*However, in slow-flowing stream segments, species like (1) water strider; (2) smallmouth bass; (3) crayfish; and (4) fingernail clams are abundant.*

Biological survey study design is of critical importance to criteria development. The design must be scientifically rigorous to provide the basis for legal action, and be biologically relevant to detect problems of regulatory concern. Since it is not financially or technically feasible to evaluate all organisms in an entire ecosystem at all times, careful selection of community components, the time and place chosen for assessments, data gathering methods used, and the consistency with which these variables are applied will determine the success of the biological criteria program. Biological surveys must therefore be carefully planned to meet scientific and legal requirements, maximize information, and minimize cost.

Biological surveys can range from collecting samples of a single species to comprehensive evaluations of an entire ecosystem. The first approach is difficult to interpret for community assessment; the second approach is expensive and impractical. A balance between these extremes can meet program needs. Current approaches range between detailed ecological surveys, biosurveys of targeted community components, and biological indicators (e.g., keystone species). Each of these biosurveys has advantages and limitations. Additional discussion will be provided in technical guidance under development.

No single type of approach to biological surveys is always best. Many factors affect the value of the approach, including seasonal variation, waterbody size, physical boundaries, and other natural characteristics. Pilot testing alternative approaches in State waters may be the best way to determine the sensitivity of specific methods for evaluating biological integrity of local waters. Due to the number of alternatives available and the diversity of ecological systems, *individuals responsible for research design should be experienced biologists with expertise in the local and regional ecology of target surface waters.* States should develop a data management program that includes data analysis and evaluation and standard operating procedures as part of a Quality Assurance Program Plan.

When developing study designs for biological criteria, two key elements to consider include (1) selecting aquatic community components that will best represent the biological integrity of State surface waters and (2) designing data collection protocols to ensure the best representation of the aquatic community. Technical guidance currently available to aid the development of study design include: *Water Quality Standards Handbook* (U.S. EPA 1983a), *Technical Support Manual: Waterbody Surveys and Assessments for Conducting Use Attainability Analyses* (U.S. EPA 1983b); *Technical Support Manual: Waterbody Surveys and Assessments for Conducting Use Attainability Analyses, Volume II: Estuarine Systems* (U.S. EPA 1984a); and *Technical Support Manual: Waterbody Surveys and Assessments for Conducting Use Attainability Analyses, Volume III: Lake Systems* (U.S. EPA 1984b). Future technical guidance will build on these documents and provide specific guidance for biological criteria development.

# Selecting Aquatic Community Components

Aquatic communities contain a variety of species that represent different trophic levels, taxonomic groups, functional characteristics, and tolerance ranges. Careful selection of target taxonomic groups can provide a balanced assessment that is sufficiently broad to describe the structural and functional condition of an aquatic ecosystem, yet be sufficiently practical to use on a daily basis (Plafkin et al. 1989; Lenat 1988). When selecting community components to include in a biological assessment, *primary emphasis should go toward including species or taxa that (1) serve as effective indicators of high biological integrity (i.e., those likely to live in unimpaired waters), (2) represent a range of pollution tolerances, (3) provide predictable, repeatable results, and (4) can be readily identified by trained State personnel.*

Fish, macroinvertebrates, algae, and zooplankton are most commonly used in current bioassessment programs. The taxonomic groups chosen will vary depending on the type of aquatic ecosystem being assessed and the type of expected impairment. For example, benthic macroinvertebrate and fish communities are taxonomic groups often chosen for flowing fresh water. Macroinvertebrates and fish both provide valuable ecological information while fish correspond to the regulatory and public perceptions of water quality and reflect cumulative environmental stress over longer time frames. Plants are often used in wetlands, and algae are useful in lakes and estuaries to assess eutrophication. In marine systems, benthic macroinvertebrates and submerged aquatic vegetation may provide key community components. Amphipods, for example, dominate many aquatic communities and are more sensitive than other invertebrates such as polychaetes and molluscs to a wide variety of pollutants including hydrocarbons and heavy metals (Reich and Hart 1979; J.D. Thomas, pers. comm.).

It is beneficial to supplement standard groups with additional community components to meet specific goals, objectives, and resources of the assessment program. Biological surveys that use two or three taxonomic groups (e.g., fish, macroinvertebrates, algae) and, where appropriate, include different trophic levels within each group (e.g., primary, secondary, and tertiary consumers) will provide a more realistic evaluation of system biological integrity. This is analogous to using species from two or more taxonomic groups in bioassays. Impairments that are difficult to detect because of the temporal or spatial habits or the pollution tolerances of one group may be revealed through impairments in different species or assemblages (Ohio EPA 1988a).

Selection of aquatic community components that show different sensitivities and responses to the same perturbation will aid in identifying the nature of a problem. Available data on the ecological function, distribution, and abundance of species in a given habitat will help determine the most appropriate target species or taxa for biological surveys in the habitat. The selection of community components should also depend on the ability of the organisms to be accurately identified by trained State personnel. Attendent with the biological criteria program should be the development of identification keys for the organisms selected for study in the biological survey.

# Biological Survey Design

Biological surveys that measure the structure and function of aquatic communities will provide the information needed for biological criteria development. Elements of community structure and function may be evaluated using a series of metrics. Structural metrics describe the composition of a community, such as the number of different species, relative abundance of specific species, and number and relative abundance of tolerant and intolerant species. Functional metrics describe the ecological processes of the community. These may include measures such as community photosynthesis or respiration. Function may also be estimated from the proportions of various feeding groups (e.g., omnivores, herbivores, and insectivores, or shredders, collectors, and grazers). Biological surveys can offer variety and flexibility in application. Indices currently available are primarily for freshwater streams. However, the approach has been used for lakes and can be developed for estuaries and wetlands.

## *Selecting the metric*

Several methods are currently available for measuring the relative structural and functional well-being of fish assemblages in freshwater streams, such as the Index of Biotic Integrity (IBI); Karr 1981; Karr et al. 1986; Miller et al. 1988) and the Index of Well-being (IWB; Gammon 1976, Gammon et al. 1981). The IBI is one of the more widely used assessment methods. For additional detail, see the "Index of Biotic Integrity" feature.

### *Index of Biotic Integrity*

*The Index of Biotic Integrity (IBI) is commonly used for fish community analysis (Karr 1981). The original IBI was comprised of 12 metrics*

- *six metrics evaluate species richness and composition*
  - *number of species*
  - *number of darter species*
  - *number of sucker species*
  - *number of sunfish species*
  - *number of intolerant species*
  - *proportion of green sunfish*
- *three metrics quantify trophic composition*
  - *proportion of omnivores*
  - *proportion of insectivorous cyprinids*
  - *proportion of piscivores*
- *three metrics summarize fish abundance and condition information*
  - *number of individuals in sample*
  - *proportion of hybrids*
  - *proportion of individuals with disease*

*Each metric is scored 1 (worst), 3, or 5 (best) depending on how the field data compare with an expected value obtained from reference sites. All 12 metric values are then summed to provide an overall index value that represents relative integrity. The IBI was designed for midwestern streams; substitute metrics reflecting the same structural and functional characteristics have been created to accommodate regional variations in fish assemblages (Miller et al. 1988).*

Several indices that evaluate more than one community characteristic are also available for assessing stream macroinvertebrate populations. Taxa richness, EPT taxa (number of taxa of the insect orders Ephemeroptera, Plecoptera, and Tricoptera), and species pollution tolerance values are a few of several components of these macroinvertebrate assessments. Example indices include the Invertebrate Community Index (ICI; Ohio EPA, 1988) and Hilsenhoff Biotic Index (HBI; Hilsenhoff, 1987).

Within these metrics specific information on the pollution tolerances of different species within a system will help define the type of impacts occurring in a waterbody. Biological indicator groups (intolerant species, tolerant species, percent of diseased organisms) can be used for evaluating community biological integrity if sufficient data have been collected to support conclusions drawn from the indicator data. In marine systems, for example, amphipods have been used by a number of researchers as environmental indicators (McCall 1977; Botton 1979; Mearns and Word 1982).

## *Sampling design*

Sampling design and statistical protocols are required to reduce sampling error and evaluate the natural variability of biological responses that are found in both laboratory and field data. High variability reduces the power of a statistical test to detect real impairments (Sokal and Rohlf, 1981). States may reduce variability by refining sampling techniques and protocol to decrease variability introduced during data collection, and increase the power of the evaluation by increasing the number of replications. Sampling techniques are refined, in part, by collecting a representative sample of resident biota from the same component of the aquatic community from the same habitat type in the same way at sites being compared. Data collection protocols should incorporate (1) spatial scales (where and how samples are collected) and (2) temporal scales (when data are collected) (Green, 1979):

- **Spatial Scales** refer to the wide variety of subhabitats that exist within any surface water habitat. To account for subhabitats, adequate sampling protocols require selecting (1) the location within a habitat where target groups reside and (2) the method for collecting data on target groups. For example, if fish are sampled only from fast flowing riffles within stream A, but are sampled from slow flowing pools in stream B, the data will not be comparable.

- **Temporal Scales** refer to aquatic community changes that occur over time because of diurnal and life-cycle changes in organism behavior or development, and seasonal or annual changes in the environment. Many organisms go through seasonal life-cycle changes that dramatically affect their presence and abundance in the aquatic community. For example, macroinvertebrate data collected from stream A in March and stream B in May, would not be comparable because the emergence of insect adults after March would significantly alter the abundance of subadults found in stream B in May. Similar problems would occur if algae were collected in lake A during the dry season and lake B during the wet season.

Field sampling protocols that produce quality assessments from a limited number of site visits greatly enhance the utility of the sampling technique. Rapid bioassessment protocols, recently developed for assessing streams, use standardized techniques to quickly gather physical, chemical, and biological quantitative data that can assess changes in biological integrity (Plafkin et al. 1989). Rapid bioassessment methods can be cost-effective biological assessment approaches when they have been verified with more comprehensive evaluations for the habitats and region where they are to be applied.

Biological survey methods such as the IBI for fish and ICI for macroinvertebrates were developed in streams and rivers and have yet to be applied to many ecological regions. In addition, further research is needed to adapt the approach to lakes, wetlands, and estuaries, including the development of alternative structural or functional endpoints. For example, assessment methods for algae (e.g. measures of biomass, nuisance bloom frequency, community structure) have been used for lakes. Assessment metrics appropriate for developing biological criteria for lakes, large rivers, wetlands, and estuaries are being developed and tested so that a multi-metric approach can be effectively used for all surface waters.

## Chapter 7

# Hypothesis Testing: Biological Criteria and the Scientific Method

Biological criteria are applied in the standards program by testing hypotheses about the biological integrity of impacted surface waters. These hypotheses include the null hypothesis—the designated use of the waterbody is not impaired—and alternative hypotheses such as the designated use of the waterbody is impaired (more specific hypotheses can also be generated that predict the type(s) of impairment). Under these hypotheses specific predictions are generated concerning the kinds and numbers of organisms representing community structure and function expected or found in unimpaired habitats. The kinds and numbers of organisms surveyed in unimpaired waters are used to establish the biological criteria. To test the alternative hypotheses, data collection and analysis procedures are used to compare the criteria to comparable measures of community structure and function in impacted waters.

*Multiple impacts in the same surface water such as discharges of effluent from point sources, leachate from landfills or dumps, and erosion from habitat degradation each contribute to impairment of the surface water. All impacts should be considered during the diagnosis process.*

## Hypothesis Testing

To detect differences of biological and regulatory concern between biological criteria and ambient biological integrity at a test site, it is important to establish the sensitivity of the evaluation. A 10 percent difference in condition is more difficult to detect than 50 percent difference. For the experimental/survey design to be effective, the level of detection should be predetermined to establish sample size for data collection (Sokal and Rohlf 1981). Knowledge of expected natural variation, experimental error, and the kinds of detectable differences that can be expected will help determine sample size and location. This forms the basis for defining data quality objectives, standardizing data collection procedures, and developing quality assurance/quality control standards.

Once data are collected and analyzed, they are used to test the hypotheses to determine if characteristics of the resident biota at a test site are significantly different from established criteria values for a comparable habitat. There are three possible outcomes:

1. The use is impaired when survey design and data analyses are sensitive enough to detect differences of regulatory importance, and significant differences were detected. The next step is to diagnose the cause(s) and source(s) of impairment.

2. The biological criteria are met when survey design and data analyses are sensitive enough to detect differences of regulatory significance, but no differences were found. In this case, no action is required by States based on these measures. However, other evidence may indicate impairment (e.g., chemical criteria are violated; see below).

3. The outcome is indeterminate when survey design and data analyses are not sensitive enough to detect differences of regulatory significance, and no differences were detected. If a State or Region determines that this is occurring, the development of study design and evaluation for biological criteria was incomplete. States must then determine whether they will accept the sensitivity of the survey or conduct additional surveys to increase the power of their analyses. If the sensitivity of the original survey is accepted, the State should determine what magnitude of difference the survey is capable of detecting. This will aid in re-evaluating research design and desired detection limits. An indeterminate outcome may also occur if the test site and the reference conditions were not comparable. This variable may also require re-evaluation.

As with all scientific studies, when implementing biological criteria, the purpose of hypothesis testing is to determine if the data support the conclusion that the null hypothesis is false (*i.e.*, the designated use is not impaired in a particular waterbody). Biological criteria cannot prove attainment. This reasoning provides the basis for emphasizing independent application of different assessment methods (e.g., chemical verses biological criteria). No type of criteria can "prove" attainment; each type of criteria can disprove attainment.

Although this discussion is limited to the null and one alternative hypothesis, it is possible to generate multiple working hypotheses (Popper, 1968) that promote the diagnosis of water quality problems when they exist. For example, if physical habitat limitations are believed to be causing impairment (e.g., sedimentation) one alternative hypothesis could specify the loss of community components sensitive to this impact. Using multiple hypotheses can maximize the information gained from each study. See the Diagnosis section for additional discussion.

# Diagnosis

When impairment of the designated use is found using biological criteria, a diagnosis of probable cause of impairment is the next step for implementation. Since biological criteria are primarily designed to detect water quality impairment, problems are likely to be identified without a known cause. Fortunately the process of evaluating test sites for biological impairment provides significant information to aid in determining cause.

During diagnostic evaluations, three main impact categories should be considered: chemical, physical, and biological. To begin the diagnostic process two questions are posed:

- What are the obvious causes of impairment?
- If no obvious causes are apparent, what possible causes do the biological data suggest?

Obvious causes such as habitat degradation, point source discharges, or introduced species are often identified during the course of a normal field biological assessment. Biomonitoring programs normally provide knowledge of potential sources of impact and characteristics of the habitat. As such, diagnosis is partly incorporated into many existing State field-oriented bioassessment programs. If more than one impact source is obvious, diagnosis will require determining which impact(s) is the cause of impairment or the extent to which each impact contributes to impairment. The nature of the biological impairment can guide evaluation (e.g., chemical contamination may lead to the loss of sensitive species, habitat degradation may result in loss of breeding habitat for certain species).

Case studies illustrate the effectiveness of biological criteria in identifying impairments and possible sources. For example, in Kansas three sites on Little Mill Creek were assessed using Rapid Bioassessment Protocols (Plafkin et al. 1989; see Fig. 4). Based on the results of a comparative analysis, habitats at the three sites were comparable and of high quality. Biological impairment, however, was identified at two of the three sites and directly related to proximity to a point source discharge from a sewage treatment plant. The severely impaired Site (STA 2) was located approximately 100 meters downstream from the plant. The slightly impaired Site (STA 3) was located between one and two miles downstream from the plant. However, the unimpaired Site (STA 1(R)) was approximately 150 meters upstream from the plant (Plafkin et al. 1989). This simple example illustrates the basic principles of diagnosis. In this case the treatment plant appears responsible for impairment of the resident biota and the discharge needs to be evaluated. Based on the biological survey the results are clear. However, impairment in resident populations of macroinvertebrates probably would not have been recognized using more traditional methods.

In Maine, a more complex problem arose when effluents from a textile plant met chemical-specific and effluent toxicity criteria, yet a biological survey of downstream biota revealed up to 80 percent reduction in invertebrate richness below plant outfalls. Although the source of impairment seemed clear, the cause of impairment was more difficult to determine. By engaging in a diagnostic evaluation, Maine was able to determine that the discharge contained chemicals not regulated under current programs and that part of the toxicity effect was due to the sequential discharge of unique effluents (tested individually these effluents were not toxic; when exposure was in a particular sequence, toxicity occurred). Use of biological criteria resulted in the detection and diagnosis of this toxicity problem, which allowed Maine to develop workable alternative operating procedures for the textile industry to correct the problem (Courtemanch 1989, and pers. comm.).

During diagnosis it is important to consider and discriminate among multiple sources of impairment. In a North Carolina stream (see Figure 5) four sites were evaluated using rapid bioassessment techni-

Figure 4.—Kansas: Benthic Bioassessment of Little Mill Creek (Little Mill Creek = Site-Specific Reference) Relationship of Habitat and Bioassessment

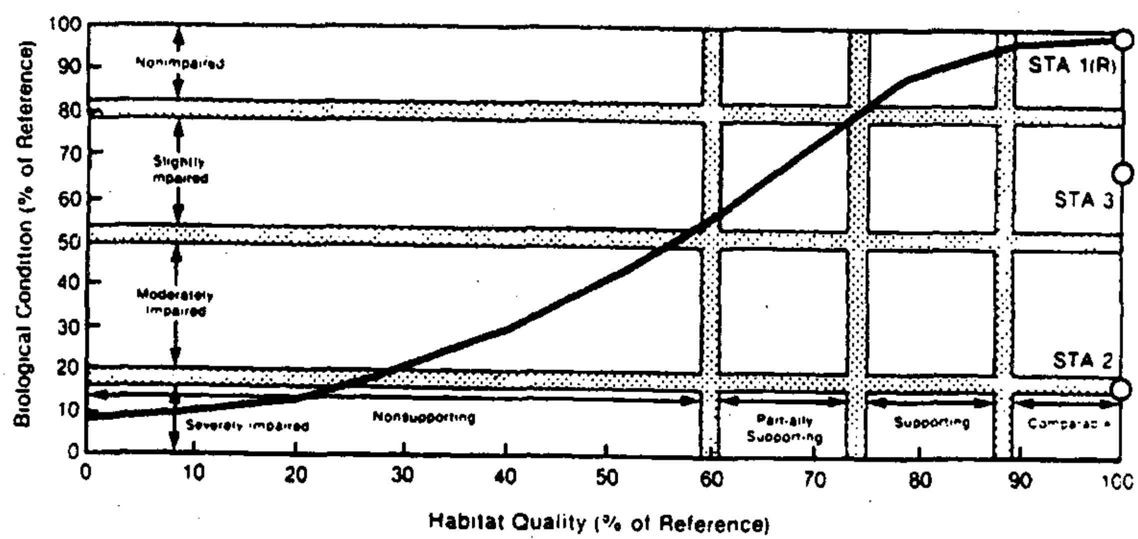



Fig 4 Three stream segments sampled in a stream in Kansas using Rapid Bioassessment Protocols (Plafkin et al 1989) [illegible] significant impairments at sites below a sewage treatment plant

**Figure 5.—The Relationship Between Habitat Quality and Benthic Community Condition at the North Carolina Pilot Study Site.**

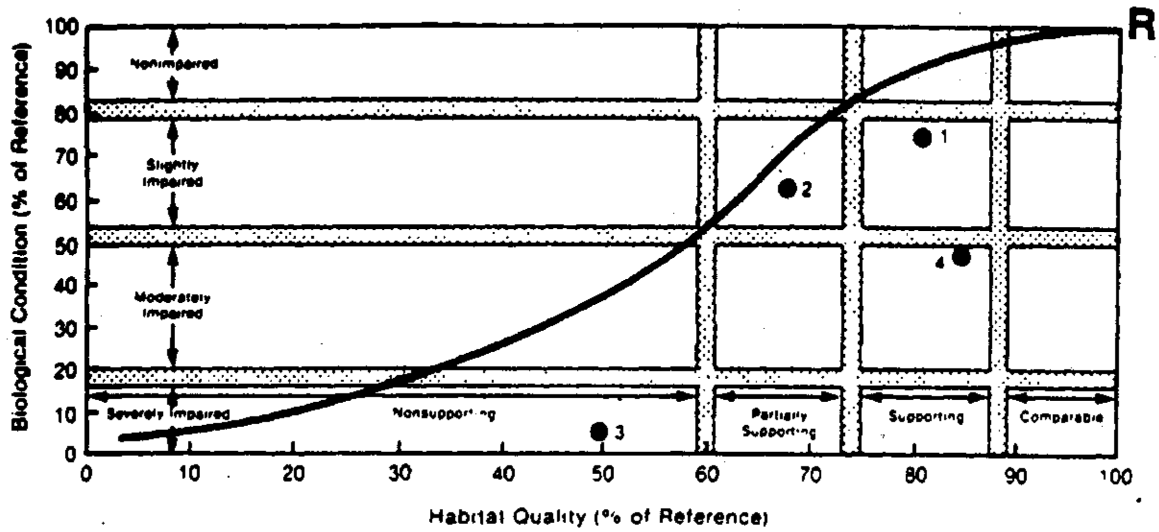



Fig. 5: Distinguishing between point and nonpoint sources of impairment requires an evaluation of the nature and magnitude of different sites in a surface water (Plafkin, et al. 1989)

ques. An ecoregional reference site (R) established the highest level of biological integrity for that stream type. Site (1), well upstream from a local town, was used as the upstream reference condition. Degraded conditions at Site (2) suggested nonpoint source problems and habitat degradation because of proximity to residential areas on the upstream edge of town. At Site (3) habitat alterations, nonpoint runoff, and point source discharges combined to severely degrade resident biota. At this site, sedimentation and toxicity from municipal sewage treatment effluent appeared responsible for a major portion of this degradation. Site (4), although several miles downstream from town, was still impaired despite significant improvement in habitat quality. This suggests that toxicity from upstream discharges may still be occurring (Barbour, 1990 pers. comm.). Using these kinds of comparisons, through a diagnostic procedure and by using available chemical and biological assessment tools, the relative effects of impacts can be determined so that solutions can be formulated to improve water quality.

When point and nonpoint impact and physical habitat degradation occur simultaneously, diagnosis may require the combined use of biological, physical, and chemical evaluations to discriminate between these impacts. For example, sedimentation of a stream caused by logging practices is likely to result in a decrease in species that require loose gravel for spawning but increase species naturally adapted to fine sediments. This shift in community components correlates well with the observed impact. However, if the impact is a point source discharge or nonpoint runoff of toxicants, both species types are likely to be impaired whether sedimentation occurs or not (although gravel breeding species can be expected to show greater impairment if sedimentation occurs). Part of the diagnostic process is derived from an understanding of organism sensitivities to different kinds of impacts and their habitat requirements. When habitat is good but water quality is poor, aquatic community components sensitive to toxicity will be impaired. However, if both habitat and water quality degrade, the resident community is likely to be composed of tolerant and opportunistic species.

When an impaired use cannot be easily related to an obvious cause, the diagnostic process becomes investigative and iterative. The iterative diagnostic process as shown in Figure 6 may require additional time and resources to verify cause and source. Initially, potential sources of impact are identified and mapped to determine location relative

**Figure 6.—Diagnostic Process**

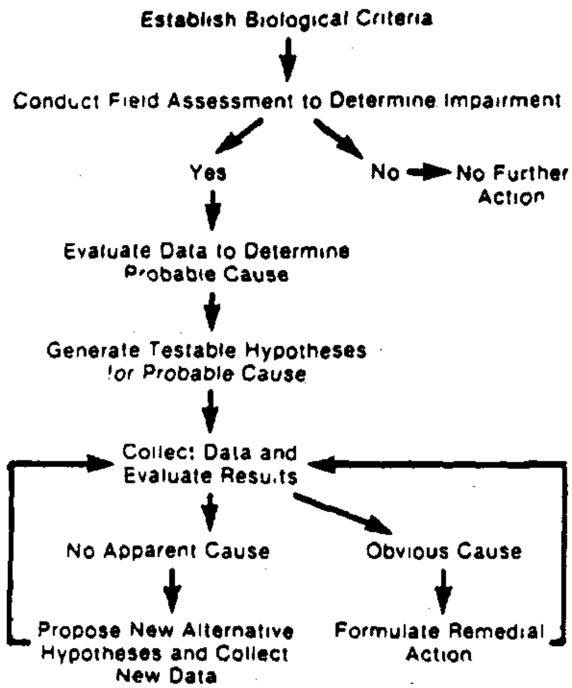



Fig. 6 The diagnostic process is a stepwise process for determining the cause of impaired biological integrity in surface waters. It may require multiple hypotheses testing and more than one remedial plan.

to the area suffering from biological impairment. An analysis of the physical, chemical, and biological characteristics of the study area will help identify the most likely sources and determine which data will be most valuable. Hypotheses that distinguish between possible causes of impairment should be generated. Study design and appropriate data collection procedures need to be developed to test the hypotheses. The severity of the impairment, the difficulty of diagnosis, and the costs involved will determine how many iterative loops will be completed in the diagnostic process.

Normally, diagnoses of biological impairment are relatively straightforward. States may use biological criteria as a method to confirm impairment from a known source of impact. However, the diagnostic process provides an effective way to identify unknown impacts and diagnose their cause so that corrective action can be devised and implemented



18626

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18627

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18628

# Appendix A

# *Common Questions and Their Answers*

**Q.** *How will implementing biological criteria benefit State water quality programs?*

**A.** State water quality programs will benefit from biological criteria because they:

a) directly assess impairments in ambient biota from adverse impacts on the environment;

b) are defensible and quantifiable;

c) document improvements in water quality resulting from agency action;

d) reduce the likelihood of false positives (i.e., a conclusion that attainment is achieved when it is not);

e) provide information on the integrity of biological systems that is compelling to the public.

**Q.** *How will biological criteria be used in a permit program?*

**A.** When permits are renewed, records from chemical analyses and biological assessments are used to determine if the permit has effectively prevented degradation and led to improvement. The purpose for this evaluation is to determine whether applicable water quality standards were achieved under the expiring permit and to decide if changes are needed. Biological surveys and criteria are particularly effective for determining the quality of waters subject to permitted discharges. Since biosurveys provide ongoing integrative evaluations of the biological integrity of resident biota, permit writers can make informed decisions on whether to maintain or restrict permit limits.

**Q.** *What expertise and staff will be needed to implement a biological criteria program?*

**A.** Staff with sound knowledge of State aquatic biology and scientific protocol are needed to coordinate a biological criteria program. Actual field monitoring could be accomplished by summer-hire biologists led by permanent staff aquatic biologists Most States employ aquatic biologists for monitoring trends or issuing site-specific permits.

**Q.** *Which management personnel should be involved in a biologically-based approach?*

**A.** Management personnel from each area within the standards and monitoring programs should be involved in this approach, including permit engineers, resource managers, and field personnel.

**Q.** *How much will this approach cost?*

**A.** The cost of developing biological criteria is a State-specific question depending upon many variables. However, States that have implemented a biological criteria program have found it to be cost effective (e.g., Ohio). Biological criteria provide an integrative assessment over time. Biota reflect multiple impacts. Testing for impairment of resident aquatic communities can actually require less monitoring than would be required to detect many impacts using more traditional methods (e.g., chemical testing for episodic events).

***Q. What are some concerns of dischargers?***

**A.** Dischargers are concerned that biological criteria will identify impairments that may be erroneously attributed to a discharger who is not responsible. This is a legitimate concern that the discharger and State must address with careful evaluations and diagnosis of cause of impairment. However, it is particularly important to ensure that waters used for the reference condition are not already impaired as may occur when conducting site-specific upstream-downstream evaluations. Although a discharger may be contributing to surface water degradation, it may be hard to detect using biosurvey methods if the waterbody is also impaired from other sources. This can be evaluated by testing the possible toxicity of effluent-free reference waters on sensitive organisms.

Dischargers are also concerned that current permit limits may become more stringent if it is determined that meeting chemical and whole-effluent permit limits are not sufficient to protect aquatic life from discharger activities. Alternative forms of regulation may be needed; these are not necessarily financially burdensome but could involve additional expense.

Burdensome monitoring requirements are additional concerns. With new rapid bioassessment protocols available for streams, and under development for other surface waters, monitoring resident biota is becoming more straightforward. Since resident biota provide an integrative measure of environmental impacts over time, the need for continual biomonitoring is actually lower than chemical analyses and generally less expensive. Guidance is being developed to establish acceptable research protocols, quality assurance/quality control programs and training opportunities to ensure that adequate guidance is available.

***Q. What are the concerns of environmentalists?***

**A.** Environmentalists are concerned that biological criteria could be used to alter restrictions on dischargers if biosurvey data indicate attainment of a designated use even though chemical criteria and/or whole-effluent toxicity evaluations predict impairment. Evidence suggests that this occurs infrequently (e.g., in Ohio, 6 percent of 431 sites evaluated using chemical-specific criteria and biosurveys resulted in this disagreement). In those cases where evidence suggests more than one conclusion, independent application applies. If biological criteria suggest impairment but chemical-specific and/or whole-effluent toxicity implies attainment of the use, the cause for impairment of the biota is to be evaluated and, where appropriate, regulated. If whole effluent and/or chemical-specific criteria imply impairment but no impairment is found in resident biota, the whole-effluent and/or chemical-specific criteria provide the basis for regulation.

***Q. Do biological criteria have to be codified in State regulations?***

**A.** State water quality standards require three components: (1) designated uses, (2) protective criteria, and (3) an antidegradation clause. For criteria to be enforceable they must be codified in regulations. Codification could involve general narrative statements of biological criteria, numeric criteria, and/or criteria accompanied by specific testing procedures. Codifying general narratives provides the most flexibility—specific methods for data collection the least flexibility—for incorporating new data and improving data gathering methods as the biological criteria program develops. States should carefully consider how to codify these criteria.

***Q. How will biocriteria fit into the agency's method of implementing standards?***

**A.** Resident biota integrate multiple impacts over time and can detect impairment from known and unknown causes. Biocriteria can be used to verify improvement in water quality in response to regulatory efforts and detect continuing degradation of waters. They provide a framework for developing improved best management practices for nonpoint source impacts. Numeric criteria can provide effective monitoring criteria for inclusion in permits.

***Q. Who determines the values for biological criteria and decides whether a waterbody meets the criteria?***

The process of developing biological criteria, including refined use classes, narrative criteria, and numeric criteria, must include agency managers, staff biologists, and the public through public hearings and comment. Once criteria are established, determining attainment\nonattainment of a use requires biological and statistical evaluation based on established protocols. Changes in the criteria would require the same steps as the initial criteria: technical modifications by biologists, goal clarification by agency managers, and public hearings. The key to criteria development and revision is a clear statement of measurable objectives.

**Q.** *What additional information is available on developing and using biological criteria?*

**A.** This program guidance document will be supplemented by the document *Biological Criteria Development by States* that includes case histories of State implementation of biological criteria as narratives, numerics, and some data procedures. The purpose for the document is to expand on material presented in Part I. The document will be available in October 1990.

A general *Biological Criteria Technical Reference Guide* will also be available for distribution during FY 1991. This document outlines basic approaches for developing biological criteria in all surface waters (streams, rivers, lakes, wetlands, estuaries). The primary focus of the document is to provide a reference guide to scientific literature that describes approaches and methods used to determine biological integrity of specific surface water types.

Over the next triennium more detailed guidance will be produced that focuses on each surface water type (e.g., technical guidance for streams will be produced during FY 91). Comparisons of different biosurvey approaches will be included for accuracy, efficacy, and cost effectiveness.

# Appendix B

# *Biological Criteria Technical Reference Guide*

## Table of Contents (*tentative*)





18632

# Appendix C

# *Biological Criteria Development by States*

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**From:** George Nichol
**To:** Rogers, Kat
**Date:** 1/24/03 3:20PM
**Subject:** Re: Dental

Should I first try and see if Darlene has a contact at DPA who writes the contract to Delta? About a year ago I did talk to a Delta person, and he said I would have to talk to someone, when the time came, that he called the "Detailer", or something like that, who wrote the dental contract from DPA to Delta Dental. But I didn't want to short-circuit the system and wanted to go to you, then Darlene, then the "Detailer" at DPA.

>>> Kat Rogers 01/24/03 02:27PM >>>
yep, it is between you and Delta at this point. Sorry. Good luck.

<<< George Nichol 1/24 1:42p >>>
Well, I guess it is time to start my next phase of my dental claim saga. As you recall I was allowed to back-pay my premiums to December 1999 after it was determined that I should have been allowed to join at that time. I did get paid for a percentage of my wife's root canal done early in 2002. However, for a bigger claim made for 2000 and 2001, the 2000 claim was not acknowledged by Delta, and the 2001 claim was refused. What is the next step I should take to Delta Dental to try to get paid? I did tell them in a letter with my original claim that I was allowed by my agency to pay the back-premiums because it was determined in 2002 that I should have been allowed to join Delta Dental in 1999.

# *APPENDIX H*

***Derivation of the 1985 Aquatic Life Criteria***

APPENDIX H

WATER QUALITY STANDARDS HANDBOOK

SECOND EDITION

18642

# *Derivation of the 1985 Aquatic Life Critera*

The following is a summary of the Guidelines for Derivation of Criteria for Aquatic Life. The complete text is found in "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses," available from National Technical Information Service - PB85-227049.

Derivation of numerical national water quality criteria for the protection of aquatic organisms and their uses is a complex process that uses information from many areas of aquatic toxicology. When a national criterion is needed for a particular material, all available information concerning toxicity to and bioaccumulation by aquatic organisms is collected, reviewed for acceptability, and sorted. If enough acceptable data on acute toxicity to aquatic animals are available, they are used to estimate the highest one-hour average concentration that should not result in unacceptable effects on aquatic organisms and their uses. If justified, this concentration is made a function of water quality characteristics such as pH, salinity, or hardness. Similarly, data on the chronic toxicity of the material to aquatic animals are used to estimate the highest four-day average concentration that should not cause unacceptable toxicity during a long-term exposure. If appropriate, this concentration is also related to a water quality characteristic.

Data on toxicity to aquatic plants are examined to determine whether plants are likely to be unacceptably affected by concentrations that should not cause unacceptable effects on animals. Data on bioaccumulation by aquatic organisms are used to determine if residues might subject edible species to restrictions by the U.S. Food and Drug Administration (FDA), or if such residues might harm wildlife that consumes aquatic life. All other available data are examined for adverse effects that might be biologically important.

If a thorough review of the pertinent information indicates that enough acceptable data exists, numerical national water quality criteria are derived for fresh water or salt water or both to protect aquatic organisms and their uses from unacceptable effects due to exposures to high concentrations for short periods of time, lower concentrations for longer periods of time, and combinations of the two.

## I. Definition of Material of Concern

A. Each separate chemical that does not ionize substantially in most natural bodies of water should usually be considered a separate material, except possibly for structurally similar organic compounds that exist only in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

B. For chemicals that do ionize substantially in most natural waterbodies (e.g., some phenols and organic acids, some salts of phenols and organic acids, and most inorganic salts and coordination complexes of metals), all forms in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different non-ionizable covalently bonded organometallic compound should usually be considered a separate material.

C. The definition of the material should include an operational analytical component. Identification of a material simply, for example, as "sodium" obviously implies "total sodium" but leaves room for doubt. If "total" is meant, it should be explicitly stated. Even "total" has different operational definitions, some of which do not necessarily measure "all that is there" in all sample. Thus, it is also necessary to reference or describe one analytical method that is intended. The operational analytical component should take into account the analytical and environmental chemistry of the material, the desirability of using the same analytical method on samples from laboratory tests, ambient water and aqueous effluents, and various practical considerations such as labor and equipment requirements and whether the method would require measurement in the field or would allow measurement after samples are transported to a laboratory.

The primary requirements of the operational analytical component are that it be appropriate for use on samples of receiving water, compatible with the available toxicity and bioaccumulation data without making overly hypothetical extrapolations, and rarely result in underprotection or overprotection of aquatic organisms and their uses. Because an ideal analytical measurement will rarely be available, a compromise measurement will usually be used. This compromise measurement must fit with the general approach: if an ambient concentration is lower than the national criterion, unacceptable effects will probably not occur (i.e., the compromise measurement must not err on the side of underprotection when measurements are made on a surface water). Because the chemical and physical properties of an effluent are usually quite different from those of the receiving water, an analytical method acceptable for analyzing an effluent might not be appropriate for analyzing a receiving water, and vice versa. If the ambient concentration *calculated* from a measured concentration in an effluent is higher than the national criterion, an additional option is to *measure* the concentration after dilution of the effluent with receiving water to determine if the measured concentration is lowered by such phenomena as complexation or sorption. A further option, of course, is to derive a site-specific criterion (1,2,3). Thus, the criterion should be based on an appropriate analytical measurement, but the criterion is not rendered useless if an ideal measurement either is not available or is not feasible.

The analytical chemistry of the material might need to be considered when defining the material or when judging the acceptability of some toxicity tests, but a criterion should not be based on the sensitivity of an analytical method. When aquatic organisms are more sensitive than routine analytical methods, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

## II. Collection of Data

A. Collect all available data on the material concerning toxicity to, and bioaccumulation by, aquatic animals and plants; FDA action levels (compliance Policy Guide, U.S. Food & Drug Admin. 1981) and chronic feeding studies and long-term field studies with wildlife species that regularly consume aquatic organisms.

B. All data that are used should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. In some cases, additional written information from the investigator may be needed. Information that is confidential, privileged, or otherwise not available for distribution should not be used.

C. Questionable data, whether published or unpublished, should not be used. Examples would be data from tests that did not contain a control treatment, tests in which too many organisms in the control treatment died or showed signs of stress or disease, and tests in which distilled or deionized water was used as the dilution water without addition of appropriate salts.

D. Data on technical grade materials may be used, if appropriate; but data on formulated mixtures and emulsifiable concentrates of the material may not be used.

E. For some highly volatile, hydrolyzable, or degradable materials, only use data from flow-through tests in which the concentrations of test material were measured often enough with acceptable analytical methods.

F. Data should be rejected if obtained by using:

- Brine shrimp — because they usually occur naturally only in water with salinity greater than 35 g/kg;
- Species that do not have reproducing wild populations in North America; or
- Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.

G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with nonresident species or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria.

## III. Required Data

A. Certain data should be available to help ensure that each of the four major kinds of possible adverse effects receives adequate consideration: results of acute and chronic toxicity tests with representative species of aquatic animals are necessary to indicate the sensitivities of appropriate untested species. However, since procedures for conducting tests with aquatic plants and interpreting the results are not as well developed, fewer data concerning toxicity are required. Finally, data concerning bioaccumulation by aquatic organisms are required only with relevant information on the significance of residues in aquatic organisms.

B. To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests (see section IV) with at least one species of freshwater animal in at least eight different families including all of the following:
   - The family Salmonidae in the class Osteichthyes.
   - A second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species, such as bluegill or channel catfish.
   - A third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.).
   - A planktonic crustacean such as a cladoceran or copepod.
   - A benthic crustacean (ostracod, isopod, amphipod, crayfish, etc.).
   - An insect (mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.).
   - A family in a phylum other than Arthropoda or Chordata, such as Rotifera, Annelida, Mollusca.
   - A family in any order of insect or any phylum not already represented.
2. Acute-chronic ratios (see section VI) with species of aquatic animals in at least three different families, provided that:
   - At least one is a fish;
   - At least one is an invertebrate; and
   - At least one is an acutely sensitive freshwater species (the other two may be saltwater species).
3. Results of at least one acceptable test with a freshwater alga or vascular plant (see section VIII). If the plants are among the aquatic organisms that are most sensitive to the material, test data on a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see section IX).

C. To derive a criterion for saltwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests (see section IV) with at least one species of saltwater animal in at least eight different families, including all of the following:
   - Two families in the phylum Chordata;
   - A family in a phylum other than Arthropoda or Chordata;
   - Either the Mysidae or Penaeidae family;
   - Three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used previously); and
   - Any other family.

2. Acute-chronic ratios (see section VI) with species of aquatic animals in at least three different families, provided that of the three species:
   - At least one is a fish;
   - At least one is an invertebrate; and
   - At least one is an acutely sensitive saltwater species (the other may be an acutely sensitive freshwater species).

3. Results of at least one acceptable test with a saltwater alga or vascular plant (see section VIII). If plants are among the aquatic organisms most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate saltwater species, if a maximum permissible tissue concentration is available (see section IX).

D. If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, derivation of a criterion might not be possible if the available acute-chronic ratios vary by more than a factor of 10 with no apparent pattern. Also, if a criterion is to be related to a water quality characteristic T (see sections V and VII), more data will be necessary.

Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it might be possible to derive a criterion if the available data clearly indicate that the Final Residue Value should be much lower than either the Final Chronic Value or the Final Plant Value.

E. Confidence in a criterion usually increases as the amount of available pertinent data increases. Thus, additional data are usually desirable.

## IV. Final Acute Value

A. Appropriate measures of the acute (short-term) toxicity of the material to a variety of species of aquatic animals are used to calculate the Final Acute Value. The Final Acute Value is an estimate of the concentration of the material, corresponding to a cumulative probability of 0.05 in the acute toxicity values for genera used in acceptable acute tests conducted on the material. However, in some cases, if the Species Mean Acute Value of a commercially or recreationally important species is lower than the calculated Final Acute Value, then that Species Mean Acute Value replaces the calculated Final Acute Value to protect that important species.

B. Acute toxicity tests should have been conducted using acceptable procedures (ASTM Standards E 729 and 724).

C. Except for tests with saltwater annelids and mysids, do not use results of acute tests during which test organisms were fed, unless data indicate that the food did not affect the toxicity of the test material.

D. Results of acute tests conducted in unusual dilution water (dilution water in which total organic carbon or particulate matter exceeded 5 mg/L) should not be used unless a relationship is developed between acute toxicity and organic carbon or particulate matter or unless data show that the organic carbon or particulate matter does not affect toxicity.

E. Acute values should be based on endpoints that reflect the total severe acute adverse impact of the test material on the organisms used in the test. Therefore, only the following kinds of data on acute toxicity to aquatic animals should be used:

1. Tests with daphnids and other cladocerans should be started with organisms less than 24-hours old, and tests with midges should be stressed with second- or third-instar larvae. The result should be the 48-hour $EC_{50}$ based on percentage of organisms immobilized plus percentage of organisms killed. If such an $EC_{50}$ is not available from a test, the 48-hour $LC_{50}$ should be used in place of the desired 48-hour $EC_{50}$. An $EC_{50}$ or $LC_{50}$ of longer than 48 hours can be used as long as the animals were not fed and the control animals were acceptable at the end of the test.

2. The result of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones should be the 96-hour $EC_{50}$ based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an $EC_{50}$ is not available from a test, the lower of the 96-hour $EC_{50}$, based on the percentage of organisms with incompletely developed shells and the 96-hour $LC_{50}$ should be used in place of the desired 96-hour $EC_{50}$. If the duration of the test was between 48 and 96 hours, the $EC_{50}$ or $LC_{50}$ at the end of the test should be used.

3. The acute values from tests with all other freshwater and saltwater animal species and older life stages of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimps, and abalones should be the 96-hour $EC_{50}$ based on the percentage of organisms exhibiting loss of equilibrium, plus the percentage of organisms immobilized, plus the percentage of organisms killed. If such an $EC_{50}$ is not available from a test, the 96-hour $LC_{50}$ should be used in place of the desired 96-hour $EC_{50}$.

4. Tests with single-celled organisms are not considered acute tests, even if the duration was 96 hours or less.

5. If the tests were conducted properly, acute values reported as "greater than" values and those above the solubility of the test material should be used because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species.

F. If the acute toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Acute Equation should be derived based on that water quality characteristic. (Go to section V.)

G. If the available data indicate that one or more life stages are at least a factor of 2 more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the Species Mean Acute Value because a species can be considered protected from acute toxicity only if all life stages are protected.

H. The agreement of the data within and between species should be considered. Acute values that appear to be questionable in comparison with other acute and chronic data for the same species and for other species in the same genus probably should not be used in calculation of a Species Mean Acute Value. For example, if the acute values available for a species or genus differ by more than a factor of 10, some or all of the values probably should not be used in calculations.

I. For each species for which at least one acute value is available, the Species Mean Acute Value should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species for which no such result is available, the Species Mean Acute Value should be calculated as the geometric mean of all available acute values — i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial concentrations of test material. (Nominal concentrations are acceptable for most test materials if measured concentrations are not available.)

NOTE: Data reported by original investigators should not be rounded off. Results of all intermediate calculations should be rounded to four significant digits.

NOTE: The geometric mean of N numbers is the $N^{th}$ root of the product of the N numbers. Alternatively, the geometric mean can be calculated by adding the logarithms of the N numbers, dividing the sum by N, and taking the antilog of the quotient. The geometric mean of two numbers is the square root of the product of the two numbers, and the geometric mean of one number is that number. Either natural (base 0) or common (base 10) logarithms can be used to calculate geometric means as long as they are used consistently within each set of data (i.e., the antilog used must match the logarithm used).

NOTE: Geometric means rather than arithmetic means are used here because the distributions of individual organisms' sensitivities in toxicity tests on most materials, and the distributions of species' sensitivities within a genus, are more likely to be lognormal than normal. Similarly, geometric means are used for acute-chronic ratios and bioconcentration factors because quotients are likely to be closer to lognormal than normal distributions. In addition, division of the geometric mean of a set of numerators by the geometric mean of the set of corresponding denominators will result in the geometric mean of the set of corresponding quotients.

J. The Genus Mean Acute Value should be calculated as the geometric mean of the Species Mean Acute Values available for each genus.

K. Order the Genus Mean Acute Value from high to low.

L. Assign ranks, R, to the Genus Mean Acute Value from "1" for the lowest to "N" for the highest. If two or more Genus Mean Acute Values are identical, arbitrarily assign them successive ranks.

M. Calculate the cumulative probability, P, for each Genus Mean Acute Value as R/(N+1).

N. Select the four Genus Mean Acute Values that have cumulative probabilities closest to 0.05. (If there are less than 59 Genus Mean Acute Values, these will always be the four lowest Genus Mean Acute Values).

O. Using the selected Genus Mean Acute Values and Ps, calculate:

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) = S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

(See original document, referenced at beginning of this appendix, for development of the calculation procedure and Appendix 2 for example calculation and computer program.)

NOTE: Natural logarithms (logarithms to base e, denoted as ln) are used herein merely because they are easier to use on some hand calculators and computers than common (base 10) logarithms. Consistent use of either will produce the same result.

P. If for a commercially or recreationally important species the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured is lower than the calculated Final Acute Value, then that geometric mean should be used as the Final Acute Value instead of the calculated Final Acute Value.

Q. Go to section VI.

V. **Final Acute Equation**

A. When enough data are available to show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be taken into account as described in section IV, steps B through G, or using analysis of covariance. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data tests. If two or more factors affect toxicity, multiple regression analysis should be used.

B. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the acute toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best documented relationship fitting these data is that between hardness and acute toxicity of metals in freshwater and a log-log relationship, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary.

C. Decide whether the data for each species are useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if based only on data for a very narrow range of water quality characteristic values. A slope based on only two data points, however, might be useful if consistent with other information and if the two points cover a broad enough range of the water quality characteristic.

In addition, acute values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic the acute values available for a species or genus differ by more than a factor of 10, probably some or all of the values should be rejected. If useful slopes are not available for at least one fish and one invertebrate, or if the available slopes are too dissimilar, or if too few data are available to adequately define the relationship between acute toxicity and the water quality characteristic, return to section IV.G, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

D. Individually for each species, calculate the geometric mean of the available acute values and then divide each of these acute values by the mean for the species. This normalizes the values so that the geometric mean of the normalized values for each species, individually, and for any combination of species is 1.0.

E. Similarly normalize the values of the water quality characteristic for each species, individually.

F. Individually for each species, perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in step B. However, now, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

G. Treat normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope, V, and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

H. For each species, calculate the geometric mean, W, of the acute toxicity values and the geometric mean, X, of the values of the water quality characteristic. (These were calculated in steps D and E.)

I. For each species, calculate the logarithm, Y, of the Species Mean Acute Value at a selected value, Z, of the water quality characteristic using the equation:

$$Y = \ln W - V(\ln X - \ln Z).$$

J. For each species, calculate the SMAV at Z using the equation:

$$SMAV = e^{Y}.$$

NOTE: Alternatively, the Species Mean Acute Values at Z can be obtained by skipping step H using the equations in steps I and J to adjust each acute value individually to Z, and then calculating the geometric mean of the adjusted values for each species individually.

This alternative procedure allows an examination of the range of the adjusted acute values for each species.

K. Obtain the Final Acute Value at Z by using the procedure described in section IV, steps J through O.

L. If the Species Mean Acute Value at Z of a commercially or recreationally important species is lower than the calculated Final Acute Value at Z, then that Species Mean Acute Value should be used as the Final Acute Value at Z instead of the calculated Final Acute Value.

M. The Final Acute Equation is written as:

$$\text{Final Acute Value} = e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])}$$

where

V = pooled acute slope

A = Final Acute Value at Z.

Because V, A, and Z are known, the Final Acute Value can be calculated for any selected value of the water quality characteristic.

## VI. Final Chronic Value

A. Depending on the data that are available concerning chronic toxicity to aquatic animals, the Final Chronic Value might be calculated in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-Chronic Ratio. In some cases, it may not be possible to calculate a Final Chronic Value.

NOTE: As the name implies, the Acute-Chronic Ratio is a way of relating acute and chronic toxicities. The Acute-Chronic Ratio is basically the inverse of the application factor, but this new name is better because it is more descriptive and should help prevent confusion between "application factors" and "safety factors." Acute-Chronic Ratios and application factors are ways of relating the acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms. Another advantage of the Acute-Chronic Ratio is that it will usually be greater than 1; this should avoid the confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

B. Chronic values should be based on results of flow- through chronic tests in which the concentrations of test material in the test solutions were properly measured at appropriate times during the test. (Exception: renewal, which is acceptable for daphnids.)

C. Results of chronic tests in which survival, growth, or reproduction in the control treatment was unacceptably low should not be used. The limits of acceptability will depend on the species.

D. Results of chronic tests conducted in unusual dilution water (dilution water in which total organic carbon or particulate matter exceeded 5 mg/L) should not be used, unless a relationship is developed between chronic toxicity and organic carbon or particulate matter, or unless data show that organic carbon, particulate matter (and so forth) do not affect toxicity.

E. Chronic values should be based on endpoints and lengths of exposure appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

1. Life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species to a different concentration of the test material throughout a life cycle. To ensure that all life stages and life processes are exposed, tests with fish should begin with embryos or newly hatched young less than 48-hours old, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young less than 24-hours old and last for not less than 21 days. Tests with mysids should begin with young less than 24-hours old and continue until seven days past the median time of first brood release in the controls.

   For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female. For mysids, data should be obtained and analyzed on survival, growth, and young per female.

2. Partial life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals in a fish species to a concentration of the test material through most portions of a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity so that all major life stages can be exposed to the test material in less than 15 months.

   Exposure to the test material should begin with immature juveniles at least two months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

3. Early life stage toxicity tests consisting of 28- to 32-day (60 days post hatch for salmonids) exposures of the early life stages of a fish species from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained and analyzed on survival and growth.

   NOTE: Results of an early life stage test are used as predictions of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a total or partial life-cycle test are available, results of an early life stage test with the same species should not be used. Also, results of early life stage tests in which the incidence of mortalities or abnormalities increased substantially near the end should not be used because these results are possibly not good predictions of the results of comparable total or partial life cycle or partial life cycle tests.

F. A chronic value can be obtained by calculating the geometric mean of the lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration in an acceptable chronic test that did not cause an unacceptable amount of adverse effect on any of the specified biological measurements and below which no tested concentration caused an unacceptable effect. An upper chronic limit is the lowest tested concentration in an acceptable chronic test that did cause an unacceptable amount of adverse effect on one or more of the specified biological measurements and above which all tested concentrations also caused such an effect.

NOTE: Because various authors have used a variety of terms and definitions to interpret and report results of chronic tests, reported results should be reviewed carefully. The amount of effect that is considered unacceptable is often based on a statistical hypothesis test but might also be defined in terms of a specified percent reduction from the controls. A small percent reduction (e.g., 3 percent) might be considered acceptable even if it is statistically significantly different from the control, whereas a large percent reduction (e.g., 30 percent) might be considered unacceptable even if it is not statistically significant.

G. If the chronic toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Chronic Equation should be derived based on that water quality characteristic. Go to section VII.

H. If chronic values are available for species in eight families as described in sections III.B.1 or III.C.1, a Species Mean Chronic Value should also be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values available for the species; appropriate Genus Mean Chronic Values should also be calculated. The Final Chronic Value should then be obtained using the procedure described in section III, steps J through O. Then go to section VI.M.

I. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio using for the numerator the geometric mean of the results of all acceptable flow-through acute tests in the same dilution water and in which the concentrations were measured. (Exception: static is acceptable for daphnids.)

For fish, the acute test(s) should have been conducted with juveniles and should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water but in a different study may be used. If no such acute tests are available, results of acute tests conducted in the same dilution water in a different laboratory may be used. If no such acute tests are available, an acute-chronic ratio should not be calculated.

J. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios available for that species.

K. For some materials, the acute-chronic ratio seems to be the same for all species, but for other materials, the ratio seems to increase or decrease as the Species Mean Acute Value increases. Thus the Final Acute-Chronic Ratio can be obtained in four ways, depending on the data available:

1. If the Species Mean Acute-Chronic ratio seems to increase or decrease as the Species Mean Acute Value increases, the Final Acute-Chronic Ratio should be calculated as the geometric mean of the acute-chronic ratios for species whose Species Mean Acute Values are close to the Final Acute Value.

2. If no major trend is apparent, and the acute-chronic ratios for a number of species are within a factor of 10, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all the Species Mean Acute-Chronic Ratios available for both freshwater and saltwater species.

3. For acute tests conducted on metals and possibly other substances with embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones (see section IV.E.2), it is probably appropriate to assume that the

acute-chronic ratio is 2. Chronic tests are very difficult to conduct with most such species, but the sensitivities of embryos and larvae would likely determine the results of life cycle tests. Thus, if the lowest available Species Mean Acute Values were determined with embryos and larvae of such species, the Final Acute-Chronic Ratio should probably be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see section XI.B)

4. If the most appropriate Species Mean Acute-Chronic Ratios are less than 2.0, and especially if they are less than 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see section XI.B).

   If the available Species Mean Acute-Chronic Ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a Final Chronic Value probably cannot be calculated.

L. Calculate the Final Chronic Value by dividing the Final Acute Value by the Final Acute-Chronic Ratio. If there was a Final Acute Equation rather than a Final Acute Value, see also section VII.A.

M. If the Species Mean Chronic Value of a commercially or recreationally important species is lower than the calculated Final Chronic Value, then that Species Mean Chronic Value should be used as the Final Chronic Value instead of the calculated Final Chronic Value.

N. Go to section VIII.

## VII. Final Chronic Equation

A. A Final Chronic Equation can be derived in two ways. The procedure described here will result in the chronic slope being the same as the acute slope. The procedure described in steps B through N usually will result in the chronic slope being different from the acute slope.

1. If acute-chronic ratios are available for enough species at enough values of the water quality characteristic to indicate that the acute-chronic ratio is probably the same for all species and is probably independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the available Species Mean Acute-Chronic Ratios.

2. Calculate the Final Chronic Value at the selected value Z of the water quality characteristic by dividing the Final Acute Value at Z (see section V.M) by the Final Acute-Chronic Ratio.

3. Use V = pooled acute slope (see section V.M) as L = pooled chronic slope.

4. Go to section VII.M.

B. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be taken into account as described in steps B through G or using analysis of covariance. The two methods are equivalent and produce identical results. The manual method described in the next paragraph provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis should be used.

C. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the chronic toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best-documented relationship fitting these data is that between hardness and acute toxicity of metals in fresh water and a log-log relationship, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section. It is probably preferable, but not necessary, to use the same transformation that was used with the acute values in section V.

D. Decide whether the data for each species are useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if founded only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, chronic values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic the chronic values available for a species or genus differ by more than a factor of 10, probably some or all of the values should be rejected.

If a useful chronic slope is not available for at least one species, or if the available slopes are too dissimilar, or if too few data are available to adequately define the relationship between chronic toxicity and the water quality characteristic, the chronic slope is probably the same as the acute slope, which is equivalent to assuming that the acute-chronic ratio is independent of the water quality characteristic. Alternatively, return to section VI.H, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

E. Individually for each species, calculate the geometric mean of the available chronic values and then divide each chronic value for a species by its mean. This normalizes the chronic values so that the geometric mean of the normalized values for each species individually, and for any combination of species, is 1.0.

F. Similarly normalize the values of the water quality characteristic for each species, individually.

G. Individually for each species, perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and the 95 percent confidence limits will be identical to those obtained in section B. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

H. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope, L, and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

I. For each species, calculate the geometric mean, M, of the toxicity values and the geometric mean, P, of the values of the water quality characteristic. (These were calculated in steps E and F.)

J. For each species, calculate the logarithm, Q, of the Species Mean Chronic Value at a selected value, Z, of the water quality characteristic using the equation:

$$Q = \ln M - L(\ln P - \ln Z).$$

NOTE: Although it is not necessary, it will usually be best to use the same value of the water quality characteristic here as was used in section V.I.

K. For each species, calculate a Species Mean Chronic Value at Z using the equation:

$$SMCV = e^{Q}.$$

NOTE: Alternatively, the Species Mean Chronic Values at Z can be obtained by skipping step J, using the equations in steps J and K to adjust each acute value individually to Z, and then calculating the geometric means of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted chronic values for each species.

L. Obtain the Final Chronic Value at Z by using the procedure described in section IV, steps J through O.

M. If the Species Mean Chronic Value at Z of a commercially or recreationally important species is lower than the calculated Final Chronic Value at Z, then that Species Mean Chronic Value should be used as the Final Chronic Value at Z instead of the calculated Final Chronic Value.

N. The Final Chronic Equation is written as:

$$\text{Final Chronic Value} = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$$

where

L = pooled chronic slope

S = Final Chronic Value at Z.

Because L, S, and Z are known, the Final Chronic Value can be calculated for any selected value of the water quality characteristic.

## VIII. Final Plant Value

A. Appropriate measures of the toxicity of the material to aquatic plants are used to compare the relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting the results of toxicity tests with plants are not well developed, results of tests with plants usually indicate that criteria which adequately protect aquatic animals and their uses will probably also protect aquatic plants and their uses.

B. A plant value is the result of a 96-hour test conducted with an alga, or a chronic test conducted with an aquatic vascular plant.

NOTE: A test of the toxicity of a metal to a plant usually should not be used if the medium contained an excessive amount of a complexing agent, such as EDTA, that might affect the toxicity of the metal. Concentrations of EDTA above about 200 µg/L should probably be considered excessive.

C. The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured, and the endpoint was biologically important.

## IX. Final Residue Value

A. The Final Residue Value is intended to prevent concentrations in commercially or recreationally important aquatic species from affecting marketability because they exceed applicable FDA action levels and to protect wildlife (including fishes and birds) that consume aquatic organisms from demonstrated unacceptable effects. The Final Residue Value is the lowest of the residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level (Compliance Policy Guide, U.S. Food & Drug Admin. 1981) for fish oil or for the edible portion of fish or shellfish, or a maximum acceptable dietary intake based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to section X because no Final Residue Value can be derived.

B. Bioconcentration Factors (BCFs) and bioaccumulation factors (BAFs) are quotients of the concentration of a material in one or more tissues of an aquatic organism, divided by the average concentration in the solution in which the organism had been living. A BCF is intended to account only for net uptake directly from water and thus almost must be measured in a laboratory test. Some uptake during the bioconcentration test might not be directly from water if the food sorbs some of the test material before it is eaten by the test organisms. A BAF is intended to account for net uptake from both food and water in a real-world situation. A BAF almost must be measured in a field situation in which predators accumulate the material directly from water and by consuming prey that could have accumulated the material from both food and water.

The BCF and BAF are probably similar for a material with a low BCF, but the BAF is probably higher than the BCF for materials with high BCFs. Although BCFs are not too difficult to determine, very few BAFs have been measured acceptably because adequate measurements must be made of the material's concentration in water to ascertain if it was reasonably constant for a long enough time over the range of territory inhabited by the organisms. Because so few acceptable BAFs are available, only BCFs will be discussed further. However, if an acceptable BAF is available for a material, it should be used instead of any available BCFs.

C. If a maximum permissible tissue concentration is available for a substance (e.g., parent material, parent material plus metabolites, etc.), the tissue concentration used in the calculation of the BCF should be for the same substance. Otherwise, the tissue concentration used in the calculation of the BCF should derive from the material and its metabolites that are structurally similar and are not much more soluble in water than the parent material.

1. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of the test material in tissue and in the test solution, and the exposure continued at least until either apparent steady state or 28 days was reached. Steady state is reached when the BCF does not change significantly over a period of time, such as 2 days or 16 percent of the length of the exposure, whichever is longer. The BCF used from a test should be the highest of the apparent steady-state BCF, if apparent steady state was reached; the highest BCF obtained, if apparent steady state was not reached; and the projected steady state BCF, if calculated.

2. Whenever a BCF is determined for a lipophilic material, the percent lipids should also be determined in the tissue(s) for which the BCF was calculated.

3. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to a BCF obtained with unaffected organisms of the same species at lower concentrations that did not cause adverse effects.

4. Because maximum permissible tissue concentrations are almost never based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight BCF by 0.1 for plankton and by 0.2 for individual species of fishes and invertebrates.

5. If more than one acceptable BCF is available for a species, the geometric mean of the available values should be used; however, the BCFs are from different lengths of exposure and the BCF increases with length of exposure, then the BCF for the longest exposure should be used.

E. If enough pertinent data exists, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

1. For each available maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife (including birds and aquatic organisms), the appropriate BCF is based on the whole body of aquatic species that constitutes or represents a major portion of the diet of the tested wildlife species.

2. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion (muscle for decapods, muscle with or without skin for fishes, adductor muscle for scallops, and total soft tissue for other bivalve molluscs) of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

F. For lipophilic materials, calculating additional residue values is possible. Because the steady-state BCF for a lipophilic material seems to be proportional to percent lipids from one tissue to another and from one species to another, extrapolations can be made from tested tissues, or species to untested tissues, or species on the basis of percent lipids.

1. For each BCF for which the percent lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a 1 percent lipid basis by dividing it by the percent lipids. This adjustment to a 1 percent lipid basis is intended to make all the measured BCFs for a material comparable regardless of the species or tissue with which the BCF was measured.

2. Calculate the geometric mean-normalized BCF. Data for both saltwater and freshwater species should be used to determine the mean-normalized BCF unless they show that the normalized BCFs are probably not similar.

3. Calculate all possible residue values by dividing the available maximum permissible tissue concentrations by the mean-normalized BCF and by the percent lipids values appropriate to the maximum permissible tissue concentrations, i.e.,

$$\textit{Residue value} = \frac{(\textit{maximum permissible tissue concentration})}{(\textit{mean normalized BCF})(\textit{appropriate percent lipids})}$$

- For an FDA action level for fish oil, the appropriate percent lipids value is 100.
- For an FDA action level for fish, the appropriate percent lipids value is 11 for freshwater criteria and 10 for saltwater criteria because FDA action levels are applied species-by-species to commonly consumed species. The highest lipid contents in the edible portions of important consumed species are about 11 percent for both the freshwater chinook salmon and lake trout and about 10 percent for the saltwater Atlantic herring.
- For a maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, the appropriate percent lipids is that of an aquatic species or group of aquatic species that constitute a major portion of the diet of the wildlife species.

G. The Final Residue Value is obtained by selecting the lowest of the available residue values.

NOTE: In some cases, the Final Residue Value will not be low enough. For example, a residue value calculated from a FDA action level will probably result in an average concentration in the edible portion of a fatty species at the action level. Some individual organisms and possibly some species will have residue concentrations higher than the mean value, but no mechanism has been devised to provide appropriate additional protection. Also, some chronic feeding studies and long-term field studies with wildlife identify concentrations that cause adverse effects but do not identify concentrations that do not cause adverse effects; again, no mechanism has been devised to provide appropriate additional protection. These are some of the species and uses that are not protected at all times in all places.

## X. Other Data

Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other adverse effect shown to be biologically important. Especially important are data for species for which no other data are available. Data from behavioral, biochemical, physiological, microcosm, and field studies might also be available. Data might be available from tests conducted in unusual dilution water (see IV.D and VI.D), from chronic tests in which the concentrations were not measured (see VI.B), from tests with previously exposed organisms (see II.F), and from tests on formulated mixtures or emulsifiable concentrates (see II.D). Such data might affect a criterion if they were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important.

## XI. Criterion

A. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

B. The Criterion Maximum Concentration (CMC) is equal to one-half the Final Acute Value.

C. The Criterion Continuous Concentration (CCC) is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value, unless other data (see section X) show that a lower value should be used. If toxicity is related to a water quality characteristic, the Criterion Continuous Concentration is obtained from the Final Chronic Equation, the Final Plant Value, and the Final Residue Value by selecting the one, or the combination, that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see section X) show that a lower value should be used.

D. Round both the Criterion Maximum Concentration and the Criterion Continuous Concentration to two significant digits.

E. The criterion is stated as follows:

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, *(1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average, and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

*where (1) = insert freshwater or saltwater

(2) = insert name of material

(3) = insert the Criterion Continuous Concentration

(4) = insert the Criterion Maximum Concentration.

## XII. Final Review

A. The derivation of the criterion should be carefully reviewed by rechecking each step of the guidelines. Items that should be especially checked are

1. If unpublished data are used, are they well documented?
2. Are all required data available?
3. Is the range of acute values for any species greater than a factor of 10?
4. Is the range of Species Mean Acute Values for any genus greater than a factor of 10?
5. Is there more than a factor of 10 difference between the four lowest Genus Mean Acute Values?
6. Are any of the four lowest Genus Mean Acute Values questionable?
7. Is the Final Acute Value reasonable in comparison with the Species Mean Acute Values and Genus Mean Acute Values?
8. For any commercially or recreationally important species, is the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured lower than the Final Acute Value?

9. Are any of the chronic values questionable?

10. Are chronic values available for acutely sensitive species?

11. Is the range of acute-chronic ratios greater than a factor of 10?

12. Is the Final Chronic Value reasonable in comparison with the available acute and chronic data?

13. Is the measured or predicted chronic value for any commercially or recreationally important species below the Final Chronic Value?

14. Are any of the other data important?

15. Do any data look like they might be outliers?

16. Are there any deviations from the guidelines? Are they acceptable?

B. On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If not, another criterion — either higher or lower — should be derived using appropriate modifications of these guidelines.