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Applicability of Ambient Toxicity Testing to National or Regional Water-Quality Assessment

By JOHN F. ELDER

U.S. GEOLOGICAL SURVEY CIRCULAR 1049

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Applicability of Ambient Toxicity Testing to National or Regional Water-Quality Assessment

By John F. Elder

Abstract

Comprehensive assessment of the quality of natural waters requires a multifaceted approach. Descriptions of existing conditions may be achieved by various kinds of chemical and hydrologic analyses, whereas information about the effects of such conditions on living organisms depends on biological monitoring. Toxicity testing is one type of biological monitoring that can be used to identify possible effects of toxic contaminants.

Based on experimentation designed to monitor responses of organisms to environmental stresses, toxicity testing may have diverse purposes in water-quality assessments. These purposes may include identification of areas that warrant further study because of poor water quality or unusual ecological features, verification of other types of monitoring, or assessment of contaminant effects on aquatic communities. Toxicity-test results are most effective when used as a complement to chemical analyses, hydrologic measurements, and other biological monitoring. However, all toxicity-testing procedures have certain limitations that must be considered in developing the methodology and applications of toxicity testing in any large-scale water-quality-assessment program.

A wide variety of toxicity-test methods have been developed to fulfill the needs of diverse applications. The methods differ primarily in the selections made relative to four characteristics: (1) test species, (2) endpoint (acute or chronic), (3) test-enclosure type, and (4) test substance (toxicant) that functions as the environmental stress.

Toxicity-test approaches vary in their capacity to meet the needs of large-scale assessments of existing water quality. Ambient testing, whereby the test organism is exposed to naturally occurring substances that contain toxicant mixtures in an organic or inorganic matrix, is more likely to meet these needs than are procedures that call for exposure of the test organisms to known concentrations of a single toxicant. However, meaningful interpretation of ambient test results depends on the existence of accompanying chemical analysis of the ambient media. The ambient test substance may be water or sediments. Sediment tests have had limited application, but they are useful because most toxicants tend to accumulate in sediments and many test species either inhabit the sediments or are in frequent contact with them. Biochemical testing methods, which have been developing rapidly in recent years, are likely to be among the most useful procedures for large-scale water-quality assessments. They are relatively rapid and simple, and more importantly, they focus on biochemical changes that are the initial responses of virtually all organisms to environmental stimuli.

Most species are sensitive to relatively few toxicants, and their sensitivities vary as conditions change. Therefore, each test method has particular uses and limitations, and no single test has universal applicability. One of the most informative approaches to toxicity testing is to combine biochemical tests with other test methods in a "battery of tests" that is diversified enough to characterize different types of toxicants and different trophic levels. However, such an approach can be costly, and if not carefully designed, it may not yield enough additional information to warrant the additional cost.

The application of toxicity tests to large-scale water-quality assessments is hampered by a number of difficulties. Toxicity tests often are not sensitive enough to enable detection of most contaminant problems in the natural environment. Furthermore, because sensitivities among different species and test conditions can be highly variable, conclusions about the toxicant problems of an ecosystem are strongly dependent on the test procedure used. In addition, the experimental systems used in toxicity tests cannot replicate the complexity or variability of natural conditions, and positive test results cannot identify the source or nature of a problem without accompanying chemical analyses. Finally, it is difficult to develop adequate control systems for toxicity tests that use ambient waters or sediments as exposure media.

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INTRODUCTION

Need for Biological Methods in Water-Quality Assessment

Protection and enhancement of water quality ultimately depend on establishment of sound management

policy on regional or national levels. Development of management policy is, in turn, dependent on regional or national programs to assess water quality—its current conditions, trends, and controlling factors. One of the particularly important and challenging needs in developing such large-scale assessment programs is appropriate planning of the collection and analysis of biological data.

There can be little doubt as to the need for biological information to accurately evaluate water-quality conditions. The terms "pollution" and "contamination" generally refer to environmental occurrence of foreign substances that are biologically detrimental. Therefore, much of the concern for water-quality degradation is biologically motivated.

The importance of biological analyses is further underscored by our understanding that water quality is not simply an expression of chemical characteristics. It is strongly influenced by biological activity, and, conversely, it strongly influences the composition and function of the biological community. For example, nitrogen and phosphorus concentrations in natural water systems are affected by uptake in algal cells (Richey, 1979; Goldman and Horne, 1983, p. 126; Schindler, 1985), and algal photosynthesis and biomass are conversely dependent on inputs of nitrogen and phosphorus (Smith, 1982; Canfield and others, 1985). Information from biological measurements often can be used to complement information from physical and chemical measurements, leading to better descriptions of water-quality conditions and improved understanding of the processes causing the conditions.

A variety of biological assessment procedures can contribute to understanding of the complex relations among biological, physical, and chemical characteristics of an ecosystem. Among the most commonly used procedures to characterize the biological aspects of water quality are measurements of

1. Distribution and abundance of floral and faunal species within an ecosystem (community surveys),
2. Biological processes, such as respiration and primary productivity, which are common indicators of community metabolic activity,
3. Biological products, such as chlorophyll and ATP (adenosine triphosphate), which also are common indicators of metabolic activity,
4. Biogeochemical processes that influence the chemical character of water and sediments,
5. Occurrence of pathogenic organisms,
6. Biological uptake and depuration of contaminants that occur in the aquatic habitat, and
7. Effects of water pollution on biota.

The results of one or more of these types of biological analyses, combined with chemical and hydrologic data, can be used to (1) define and quantify biological processes that affect physical and chemical aspects of water quality, (2) determine the sanitary quality of the water, (3) determine the occurrence, distribution, and fate of contaminants, and (4) assess the relation between the physical and chemical factors and the functional or structural aspects of the biological community.

Difficulties of Biological Methods in Water-Quality Assessment

Notwithstanding the obvious need for implementation of biological procedures in large-scale studies of water quality, it is clear that there are particular problems that are likely to be associated with biological water-quality-assessment work. The heterogeneous nature of biological systems is among the most important of such problems. Biological variables can fluctuate widely over space and time and are influenced by innumerable physical, chemical, and ecological factors (Hutchinson, 1953; Odum, 1969; Wallen and Botek, 1984). Furthermore, species distributions are extremely patchy (nonuniform), even within a single ecosystem (Odum, 1971, p. 205), and certainly over broad geographical areas. Different species respond very differently to particular environmental stimuli or stresses (Luoma, 1977). Biological variability severely limits universal applicability of native bioindicator organisms. It becomes very difficult to separate the effects of contaminants from natural variation, especially in comparisons among different aquatic systems.

Problems of methodology are important considerations in developing a biomonitoring program. Some biomonitoring methods are not well defined, tested, or verified. This is partly due to the biological variability and nonuniform species distribution already mentioned. For some types of analyses (toxicity tests or biogeochemical process measurements, for example), it is extremely difficult to establish standardized procedures to be used in a consistent manner throughout a large-scale program. Even if a satisfactory procedure is available, the cost of applying it widely throughout a region can be prohibitive. Many types of biological analyses are labor intensive. This is especially true for large-scale assessments, because natural variability requires that large amounts of data be collected to compensate for the variability.

Purpose and Scope

This report examines toxicity testing—just one of the different types of biological measurement that might be used for evaluation of water quality. The overall purpose of the report is to evaluate the utility and feasibility of current toxicity-test methods for ambient water-quality assessments conducted on regional or national scales. Toxicity testing has been used widely in specialized research projects, but certain limitations of current procedures cast some doubt on whether it can be applied successfully to large-scale water-quality assessments.

Specific questions addressed in this report include the following:

1. What are the characteristics and applications of different types of toxicity tests?
2. What are the advantages and disadvantages of different types of test procedures, particularly with reference to application in large-scale water-quality assessments?
3. Do the results of toxicity tests accurately reflect environmental conditions and the probable effects of contaminants on biota in natural systems?
4. Will different toxicity tests result in different conclusions about existing toxicant problems in the environment?
5. Is there a particular type of test, with respect to selection of test species, test substance (ambient or artificial), and test medium (water or sediment), that can be applied in standardized format, with consistently reliable results, over a broad range of aquatic systems and environmental conditions?

The evaluation of toxicity testing for water-quality assessment is based largely on review of existing information. This information includes background data about the current status of toxicological methods and toxicity-test results from published aquatic toxicological studies. Various types of toxicity-test designs are discussed, and criteria for selection of test organisms and testing procedures are identified.

A great deal of information about procedures and applications of aquatic toxicity tests has been published in reports and technical papers in scientific literature (Kline and others, 1987). It is not the purpose of this report to review this literature exhaustively. Instead, the objectives are to summarize important concepts and conclusions that are contained in many past and current reports on toxicity-test applications and to consider the implications of these concepts for possible application of the methodology in large-scale projects.

Detailed descriptions of methods also may be found in the literature. The appendix identifies some of these sources and includes a discussion of general methodological principles.

The term "toxicity test" as discussed in this report refers to any water-quality-assessment procedure that involves monitoring of responses of organisms to environmental stresses after exposure of the organisms to such stresses either in the natural environment or in controlled enclosures. The effects of the stresses are evaluated by monitoring an "endpoint" response. The endpoint may be mortality, or it may be a sublethal response. Toxicity tests have been used frequently in a wide variety of studies of pollutant impacts on aquatic systems.

An "ambient" toxicity test is one in which the stress on the test organism is produced by exposure to a natural water or sediment sample, or an extract of such a sample. This differs from a more controlled experimental situation in which the test organisms are exposed to known concentrations of specific toxic agents. Ambient testing would be the method of choice if the results are to be used for assessment of existing water or sediment quality.

The term "bioassay" commonly is used interchangeably with "toxicity test" in aquatic toxicological studies. Technically, the terms are not synonymous (Murty, 1986, p. 117). A toxicity test is used to determine the toxicity of an agent to a test species. A bioassay test, like a chemical test, is used to measure the concentration of a chemical or effluent, using biological-response intensity as a means of quantification. By these definitions, "toxicity test" more closely signifies the procedure that is appropriate for a water-quality assessment in which ambient materials are examined for possible content of toxic agents. Hence, "toxicity test" is the preferred term throughout the remainder of this report.

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BENEFITS AND LIMITATIONS OF TOXICITY TESTING

Benefits

Toxicity tests show directly how certain organisms respond to contaminants under certain conditions. They supply complementary data that can help fill some of the information gaps left by chemical analyses.

There are some very compelling arguments for the use of toxicity tests in assessment of water quality (Chapman and Long, 1983). Regardless of what levels of contaminants are found in the environment, their effects on biota are unknown without some biological measurements. Furthermore, chemical analyses, no matter how extensive, cannot include measurement of all possible toxic agents that may occur in the system. Not only do toxicity tests show the biological effects of specific contaminants, but they also integrate the effects of combinations of contaminants, including those that are not detected by established analytical methods.

In most cases of environmental contamination, more than one toxic substance is present at concentrations greater

than background levels. The effects of combinations of toxic substances are likely to be different than the sum of their individual effects (Voyer and Heltshe, 1984). In cases of synergism, the total effect is greater than individual toxicities would suggest (Macek, 1975; Thompson and others, 1980; Hermens and others, 1984). Conversely, where antagonism occurs, the total effect is smaller than might be caused by the substances' individual effects (Bartlett and others, 1974; Christensen and others, 1979; Hemelraad and others, 1987). Sequential exposure to two or more toxicants may sensitize biota so that they are more susceptible to damage after the initial exposure (Trevors and others, 1982). Conversely, the initial stress may stimulate protective mechanisms, such as production of metallothionein-like binding agents, that decrease the organism's susceptibility to further damage upon exposure to additional toxicants (Doherty and others, 1987). Mere detection of the toxicants reveals nothing about these kinds of interactions.

Occurrences of environmental contaminants are further complicated by nonuniform spatial or temporal distribution. Intermittent releases into the environment may occur, especially from point sources that discharge directly to the affected ecosystem (Elder and Dresler, 1988). Water concentrations of pollutants are especially subject to temporal variability because the water is mobile and contaminant inputs tend to be transported or diluted quickly. Sediments, as historical integrators of water quality (Feltz, 1980), tend to accumulate substances from the overlying water, and are much less prone to show short-term temporal fluctuations in contaminant concentrations. However, sediments are likely to show considerable spatial variability of contaminant concentrations (Salomons and Forstner, 1984, p. 165). Chemical detection of contaminants is thus highly dependent on sampling time and frequency (in the case of water and suspended sediments) and sampling location (in the case of sediments). Certain toxicity tests, primarily those that are conducted in situ, may diminish this problem by integrating effects over time and space.

Another reason for use of toxicity testing in water-quality assessment is the limited capability of chemical analysis to detect specific forms and degradation products of metals and organic compounds. Total toxicant concentration data can be misleading because the toxic effects can vary enormously depending on the speciation of the chemical (Diks and Allen, 1983; Mayes and others, 1985). Furthermore, chemical analyses may not show the products of degradation, which are likely to have different toxicological effects than those of the parent compounds (Mayes and others, 1985).

Limitations

There also are limitations to the use of toxicity tests. Principal among these is that it is extremely difficult, if not impossible, for toxicity-test models to truly mimic natural systems. Therefore, responses of selected test organisms to contaminants in a controlled environment are unlikely to represent accurately the responses of a complex natural community to the same contaminant. Furthermore, it is unlikely that the biota in the natural system would be presented with the same simplicity of exposure that is characteristic of the regulated and relatively constant conditions of a toxicity test. Because of this weakness, toxicity tests have questionable predictive value, and may even be misleading.

Another limitation of toxicity testing is the difficulty of identifying cause-effect relations. Even if a test demonstrates toxicological responses of biota exposed to ambient water or sediments, it cannot identify the substances or their concentrations that cause such responses. Chemical analyses are needed to identify possible toxic agents that are present in the system. The coupling of biological and chemical monitoring procedures to obtain complementary data has been effective in some studies (Pessah and Cornwall, 1980; Long and Chapman, 1985). However, identification of contaminant occurrence at elevated concentrations and simultaneous observation of abnormal responses of biota in bioassay tests do not necessarily demonstrate cause-effect relations.

Most toxicity tests are conducted in enclosures outside the natural aquatic environment. Many of the physical and chemical conditions within the test enclosures are controlled. Factors such as temperature, salinity, water hardness, pH, and photoperiod may vary from study to study. In some cases they are set to be consistent with conventional experimental methods. In other cases they are set to mimic, as closely as possible, the natural environmental conditions of the test species. Control of the test conditions is needed in order to interpret the results. However, the ambient conditions are likely to have a significant effect on test results (Leeuwangh, 1978; Judy and Davies, 1979; Graney and others, 1984; Babich and Stotzky, 1985). Variability of uncontrolled test factors, such as bacterial activity, chemical speciation, and health of the test organisms, may increase further the variability of test results.

Another cause of response variability is the wide variance of different species in their sensitivities and responses to any particular toxic substance (Plotkin and Ram, 1984; Phipps and Holcombe, 1985; Slooff, 1985). Even within a single species, there may be significant differences in sensitivities among individuals of different sexes, age groups, and genotypes (Adelmar and Smith, 1976; Wright and Frain, 1981; Nebeker, Cairns, and Wise, 1984; Woltering, 1984; Nebeker and others, 1985). Such biological nonuniformity, compounded with the variability due to test conditions, makes it very difficult to compare results from different studies.

Toxicity-test methodology generally calls for relatively standard formats for evaluating biological responses. In particular, the standard endpoints are concentrations that, in a specified time period, produce mortality in half the tested population ("50-percent lethal concentration," or LC50) or elicit an observable response in half the tested population ("50-percent effective concentration," or EC50). White and Champ (1984) criticized these endpoints, stating that they are arbitrarily chosen for the convenience of reporting results and have no demonstrated relevance to true hazard levels in the natural environment. Because of the dependence on test conditions, the 50-percent effective dose level may vary over several orders of magnitude. Hence, the toxicity-test results may have limited broad-scale significance for human health or environmental preservation. However, despite the implications of their title—"The great bioassay hoax, and alternatives"—White and Champ (1984) did not demonstrate total uselessness of toxicity-test methods or applications. The authors did not deny that use of biological indicators can be a valuable tool to complement other kinds of data in an evaluation of environmental contamination. In fact, they suggested that toxicity studies can be designed and implemented so that they are useful, provided they meet the critical criteria of scientific soundness, adequate relation to natural systems, and relation to broad-scale processes.

The practical and logistical difficulties of toxicity testing can be considerable. Most tests require an elaborate laboratory setup and specially trained personnel. Test organisms are often reared in captivity, which may itself introduce variables that can affect experimental results (Ten Berge, 1978; Goulden and others, 1982). Applications of toxicity-test procedures over a broad geographical area to assess regional or national water-quality problems require either the operation of numerous laboratories in different areas or transport of samples to a central processing laboratory. Either option poses logistical problems.

One of the major difficulties with ambient tests is the establishment of control systems. Wong has pointed out (1984) that "a control medium can never be obtained since we can neither remove contaminants from ambient waters nor can we simulate water with identical chemistry." Even if simulation could be achieved, the conditions in ambient media are not static, and it would be impossible to simulate natural fluctuations. The usual solution to this problem is to avoid control systems altogether and depend either on serial dilutions of the ambient media (De Vries and Hotting, 1985; Gaur and Kumar, 1986) or on comparisons among samples from different sites (Long and Chapman, 1985; Mount and Norberg-King, 1985; Norberg-King and Mount, 1986) to evaluate relative toxicity.

The limited capacity of toxicity testing to predict ecological effects of toxic agents within a complex and variable aquatic ecosystem was emphasized by Stumm and others (1983). The authors stressed a need to consider various processes, such as adsorption, atmospheric exchange, microbial degradation, and chemical transformation, that affect the chemistry and biological availability of toxicants. They suggested that toxicity testing, even if combined with chemical monitoring, is not enough; meaningful information about environmental cycling of contaminants depends on modeling based on data that describe compound-specific variables (including solubility, vapor pressure, and lipophility), transformation processes, and spatial and temporal distribution of contaminants in the natural environment.

Special Considerations for Large-Scale Toxicity Testing

Toxicity testing on a regional or national scale would have special requirements distinct from those of tests conducted as part of small-scale, specialized studies. The most important consideration is that tests would be applied to a wide diversity of sample sites. Many different contaminants would be encountered; hence, tests would not be aimed at particular toxic compounds or elements. Environmental variables and biological communities also would vary over broad ranges among different sites. There would be little value in designing a test that is representative of a particular community type because it would then fail for other community types. Single-species tests would have limited capacity to represent the diverse communities characteristic of the sample sites.

The most important function of toxicity tests in a large-scale program would be to identify sites where indications of toxicity coincide with contaminant problems suggested by results of analyses and any other biological monitoring that may be done at the sites. The tests could serve as initial indicators, in which the results of tests at any given site may determine whether or not more detailed monitoring or research at the site is advisable.

In addition to being diverse, most of the sample sites would be free of severe contamination. To assess the quality of usable waters, the emphasis would be on natural waters rather than on effluents, leachates, or other directly contaminated materials. For a toxicity test to be useful on natural water and sediment samples, it must be sensitive to relatively low concentrations of at least some contaminants. At the same time, the test should not be so complex, time consuming, or expensive that it could not be conducted on a large number of samples from widely dispersed locations.

The broad geographical distribution of study sites in a large-scale project would almost certainly require shipment of samples to a central laboratory for analysis. There would be a need to test for possible changes in toxicity characteristics of the samples during shipment.

USES OF TOXICITY-TEST RESULTS

Some possible uses of toxicity tests are shown in table 1. The uses are listed in order of the probable risk of error; however, the absolute risk may vary considerably among different situations, owing to different kinds of restrictions presented by different cases. It is impossible to eliminate the risk of error entirely. Hence, there is always a dilemna in designing or interpreting a test. If the test is overextended (more is interpreted from the test results than the data can support), the amount of information produced may be impressive but there is substantial risk that much of it is erroneous. If the test is underextended, the risk of error is low but the amount of information generated may be so minor that the test was hardly worth the effort.

Toxicity-test results provide information on the toxicity of particular contaminants to particular organisms under particular conditions. This can be valuable information if used in the proper context. However, extrapolation of the results to more general conditions may lead to erroneous or misleading interpretations. A few studies have demonstrated some of the difficulties in extrapolating toxicity-test results to predict toxicant effects in situations other than the specific tested case. Nevertheless, a certain amount of extrapolation may be valid. Chapman (1983) emphasized that existing laboratory toxicity-test data generally are inapplicable as precise indicators of toxic-effect levels in nature, although they have considerable capability for answering site-specific questions. Interspecific variation in sensitivity to toxicants should discourage most attempts to extrapolate results to nontested species. Nevertheless, LeBlanc (1984) pointed out that closely related species have similar sensitivities to most chemicals. It is reasonably safe to assume, for example, that a substance that produces a toxic response in bluegills will also have some toxic effect on large-mouth bass, but it probably would be invalid to assume similar toxicity to invertebrate species based solely on the bluegill results. As the breadth of extrapolation increases, so does the risk.

Uses of toxicity-test results are determined in part by recognition of limitations of the tests balanced against the needs and possible benefits of the tests. Because of the limitations, some water-quality researchers may be discouraged from including any kind of toxicity-testing procedures in their investigations. On the other hand, if toxicity-testing procedures are to be included, full awareness of their limitations will enable the researchers to minimize the detrimental effects of these limitations.

Some of the important potential problems of toxicity testing can be avoided or diminished by initiating the study with a clear perception and statement of its purpose. The stated purpose should be adequately restrictive with respect to the possible applications of the test results shown in table 1 so that the test is not overextended. In addition, the purpose should be suited to the needs and constraints of the investigation and the study area. An appropriate statement of purpose, followed by execution of the study such that it fulfills the purpose, will do a great deal to minimize misinterpretation and perceptions that the test results are irrelevant or unimportant.

Table 1. Some possible uses of toxicity tests

[Listed in order of increasing probable risk of error]

1.	Identification of toxic conditions in waters or sediments without describing effects of those conditions
2.	Verification of other assessment measurements
3.	Assessment of effects of toxic conditions on one or a few test species
4.	Prediction of effects of toxic conditions on one or a few test species
5.	Assessment of effects of toxic conditions on entire communities
6.	Prediction of effects of toxic conditions on entire communities
7.	Establishment of environmental standards

PROCEDURES AND APPLICATIONS

A wide variety of toxicity-test methods have been developed to fulfill the needs of diverse applications. Each test has particular purposes and limitations, and no test is universally applicable. The test methods may be distinguished primarily on the basis of four characteristics: (1) test species, (2) endpoint (acute or chronic, and variations of each), (3) test enclosure, and (4) test substance (toxicant) that acts as the environmental stress. Some aspects of each of these design characteristics are discussed here.

Test-Species Selection

The most important feature that distinguishes different toxicity-test methods is the selection of plant or animal species to be used as indicators of contaminant effects. This is a necessary early step in nearly all toxicity-test procedures. Because of the difficulty of testing toxicity responses of all potentially affected organisms in the natural water body of interest, one or a small number of bioindicator species generally is used to represent a larger community.

The selection of test species usually is based on several criteria related to the reliability of the organisms as indicators and the feasibility of their use as captive organisms. Various authors have discussed important requirements for a species to be useful as a toxicity-test organism (Benfield and Buikema, 1980; Phillips, 1980; Nebeker, Cairns, Gakstatter, and others, 1984). Some requirements, or criteria for species selection, are shown in table 2, listed

Table 2. Criteria for species selection

[Listed in order of decreasing estimated overall importance]

1.	Sensitivity: The organism should respond to a variety of contaminants, at concentrations that may be encountered in the natural environment and with an intensity of response that is related to contaminant concentration(s).
2.	Representativeness: The organism should respond to the contaminant in ways that characterize responses that could be expected from a large number of other species. It should not be prone to giving false positive or false negative results.
3.	Response detection: Responses or endpoints should be readily detectable and quantifiable. If life-cycle tests are used, life stages should be easy to identify.
4.	Amenability to laboratory culture: The organism should be adaptable to laboratory captivity without presenting unusual problems for rearing or experimentation. Control mortality should not be a problem.
5.	Reproducibility of results: Repeated experiments should give uniform results, within acceptable error limits. There should not be a great deal of variability among individuals in their responses to contaminants.
6.	Relevance: The organism should have ecological or economic significance because of its abundance, importance in the food web, or commercial importance.
7.	Simplicity of test: Toxicity-testing procedures should be simple and rapid.
8.	Short-duration life cycle: If life-cycle testing is to be done, the cycle should be short so that tests can be completed in reasonable time.
9.	Availability of background information: A data base of toxicity information, based on results from previous work, should be available.
10.	Documented methodology: There should be established and tested procedures for use of the species in toxicity tests.
11.	Biological uptake activity: The contaminant cannot directly affect the organism if it is not incorporated by the organism in some way, either internally or externally. Therefore, bioaccumulation or uptake rates should be relatively rapid.
12.	Low cost: Toxicity-testing procedures with the species should not preclude accomplishing a meaningful number of analyses.

in order of estimated importance. Any species that does not meet the description given for a particular criterion is less than ideal as a test species with respect to that criterion.

Among the great variety of aquatic floral and faunal species, a relatively small number have emerged as favorites in toxicological research. Most species simply do not meet enough of the requirements listed in table 2 to be considered as test organisms. Even among those that are acceptable, none of them would be considered exceptional with respect to all 12 criteria. Each species has particular characteristics that limit its use in certain applications, and no species clearly stands out as a "universal" indicator.

Although the criteria are listed in table 2 in order of estimated overall importance, the priorities of a specific study may alter this order considerably. Hence, species selection depends to a large extent on the peculiarities and objectives of the study.

Most of the species commonly used in freshwater toxicity tests discussed later in this report are listed in tables 3 and 4. Their taxonomic lineages are shown in figure 1. A wide variety of taxonomic groups and trophic levels is represented. This variety of usable species enhances the potential usefulness of toxicity testing for characterizing aquatic communities; selection of a few test species may provide information about toxicant effects for a broad spectrum of organisms in the community. However, because of practical and economic considerations, the scope of most studies is limited to one or two test species.

Certain characteristics of taxonomic groups and individual species, including habitat, trophic level, economic importance, and tolerance ranges for environmental variables, influence the selection of toxicity-test species. Information about these characteristics is given in tables 3 and 4. More detailed information can be found in the reference publications listed at the end of table 4.

Species selections are made by toxicity-test researchers for various reasons. The reasons are not given in most published reports, especially if the species is well known as a test organism. If there is an established precedent of its use for toxicity testing, then there is generally an implied assumption that its use is appropriate for the particular study being reported. However, many investigators do give explicit reasons for their choices of test species. Categorized in tables 5–10 are statements about particular species selections in a variety of published toxicity-test studies.

The information shown in tables 5–10 originally was assembled to look for patterns of strengths and weaknesses of different species. For each species, it was expected that authors would observe similar advantages and disadvantages with respect to a particular set of criteria. In other words, species were expected to be distinguishable in their patterns of strengths and weaknesses. In fact, the tables show little consistency in how species were rated on a particular criterion. There was considerable overlap in stated advantages and disadvantages of species or related groups of species. For example, amenability to laboratory culture (criterion 4) was considered an advantage of *Daphnia magna* by several authors (table 7), but an almost equal number of authors stated that this species was quite difficult to culture. *Pimephales promelas* (table 9) was selected for many studies because of sensitivity, as expected, but a number of other reasons were given, and no clear pattern emerged about which of those might be most important. Criterion 6 (relevance) might be expected to be an important reason for selecting any fish species, but tables 9 and 10 do not give a strong suggestion that this criterion was more critical than many other possible reasons for selection.

Results in tables 5–10 indicate that certain criteria for test-species selection were considered much more frequently than others. Sensitivity (criterion 1) was nearly always considered, whereas other factors, such as response-detection capability (criterion 3) or biological-uptake activity (criterion 11), were discussed infrequently. Therefore, it is difficult to achieve a balanced view of the strengths and weaknesses of each species. Whatever the species selected, different authors tend to give the same reasons for their selection, although there may be other important reasons that were not considered or mentioned.

Table 3. Characteristics of some floral and faunal species frequently used in aquatic

[Except for fish, common names are very general or nonexistent ("spp." indicates that various or unnamed indicated by "o"; absence of "o" signifies that the characteristic does not apply or that information is not

			Wide tolerance range[1]				Predominant trophic level					
Taxonomic group	Scientific name	Common name	pH	Temp.	D.O.	Salinity	Decomposer	Autotroph	Heterotroph	Herbivore	Carnivore	Omnivore
Bacteria	Photobacterium phosphoreum			o			o					
	Spirillum voluntans			o			o					
	Pseudomonas spp.			o			o					
	Aeromonas hydrophila			o			o					
Protozoans	Chilomonas paramecium	paramecium	o	o					o			
Green algae	Selenastrum capricornutum			o				o				
	Scenedesmus quadricauda			o				o				
	Chlorella stigmatophora			o				o				
	Stigeoclonium tenue			o				o				
Macrophytes	Lemna spp.	duckweed						o				
	Eichhornia crassipes	water hyacinth						o				
Nematodes	Panagrellus redivivus								o			
	Panagrellus silusiae								o			
Oligochaetes	Limnodrilus hoffmeisteri		o	o		o			o			
	Tubifex tubifex	sludge worm	o	o	o	o			o			
	Lumbriculus variegatus								o			
	Nais spp.		o						o			
	Ilyodrilus spp.		o			o			o			
Cladocerans	Daphnia magna	water flea	o	o						o		
	Daphnia pulex	"	o	o						o		
	Daphnia pulicaria	"	o	o						o		
	Daphnia laevis	"	o	o						o		
	Ceriodaphnia reticulata	"	o	o						o		
Amphipods	Gammarus lacustris	scud										o
	Gammarus pulex	"										o
	Hyallela azteca	"										o
Decapods	Orconectes immunis	crayfish										o
Insects	Chironomus tentans	midge	o	o	o					o		
	Tanytarsus spp.	tanytarsus	o	o	o					o		
	Hexagenia limbata	mayfly		o								o
Mollusks	Corbicula fluminea (or manilensis)	Asiatic clam										o
	Musculium transversum	fingernail clam										o
	Anodonta cygnea											o
Fish	Pimephales promelas	fathead minnow										o
	Salmo gairdneri	rainbow trout									o	
	Salmo trutta	brown trout									o	
	Salmo clarki	cutthroat trout									o	
	Salvelinus fontinalis	brook trout									o	
	Lepomis macrochirus	bluegill										o
	Carassius auratus	goldfish										o
	Ictalurus punctatus	channel catfish										o
	Cyprinodon variegatus	sheepshead minnow										o

[1]Relative to other toxicity-test species.

[2]Nonmobile: have no anatomical structures for locomotion; subject to transport by currents and waves.

[3]Epibenthic: lives and may move about on surface of sediments. Infauna: burrows beneath sediment surface.

[4]Asexual reproduction may occur, but is not necessarily the only means of reproduction.

[5]Supports other economically important populations by ecological association, such as serving as food supply or creating shelter.

species of this genus are used). Characteristics of adult forms, based on the best information available, are available. "L" signifies larval or immature forms only. D.O., dissolved oxygen; temp., temperature]

Scientific name	Habitat					Reproduction		Economic importance		
	Water			Benthic[3]		Short life cycle[1]	Asexual[4]	Human food source	Support of resource[5]	Detrimental or nuisance
	Non-mobile[2]	Weakly mobile	Very mobile	Epi-benthic	In-fauna					
Photobacterium phosphoreum	o					o	o			
Spirillum voluntans	o					o	o			
Pseudomonas spp.	o					o	o			
Aeromonas hydrophila	o					o	o			
Chilomonas paramecium		o				o	o			
Selenastrum capricornutum	o					o	o			
Scenedesmus quadricauda	o					o	o			
Chlorella stigmatophora	o					o	o			
Stigeoclonium tenue	o					o	o			
Lemna spp.	o								o	o
Eichhornia crassipes	o								o	o
Panagrellus redivivus					o		o			
Panagrellus silusiae					o		o			
Limnodrilus hoffmeisteri					o					
Tubifex tubifex					o					
Lumbriculus variegatus					o					
Nais spp.					o					
Ilyodrilus spp.					o					
Daphnia magna		o					o		o	
Daphnia pulex		o					o		o	
Daphnia pulicaria		o					o		o	
Daphnia laevis		o					o		o	
Ceriodaphnia retic		o				o	o		o	
Gammarus lacustris				o					o	
Gammarus pulex				o					o	
Hyallela azteca				o					o	
Orconectes immunis		o		o	o			o	o	
Chironomus tentans				L					o	o
Tanytarsus spp.				L					o	o
Hexagenia limbata				L					o	o
Corbicula fluminea (or manilensis)		L		o						o
Musculium transversum				o					o	
Anodonta cygnea				o					o	
Pimephales promelas			o					o		
Salmo gairdneri			o					o	o	
Salmo trutta			o					o	o	
Salmo clarki			o					o	o	
Salvelinus fontinalis			o					o	o	
Lepomis macrochirus			o					o	o	
Carassius auratus			o						o	
Ictalurus punctatus			o					o	o	
Cyprinodon variegatus			o						o	

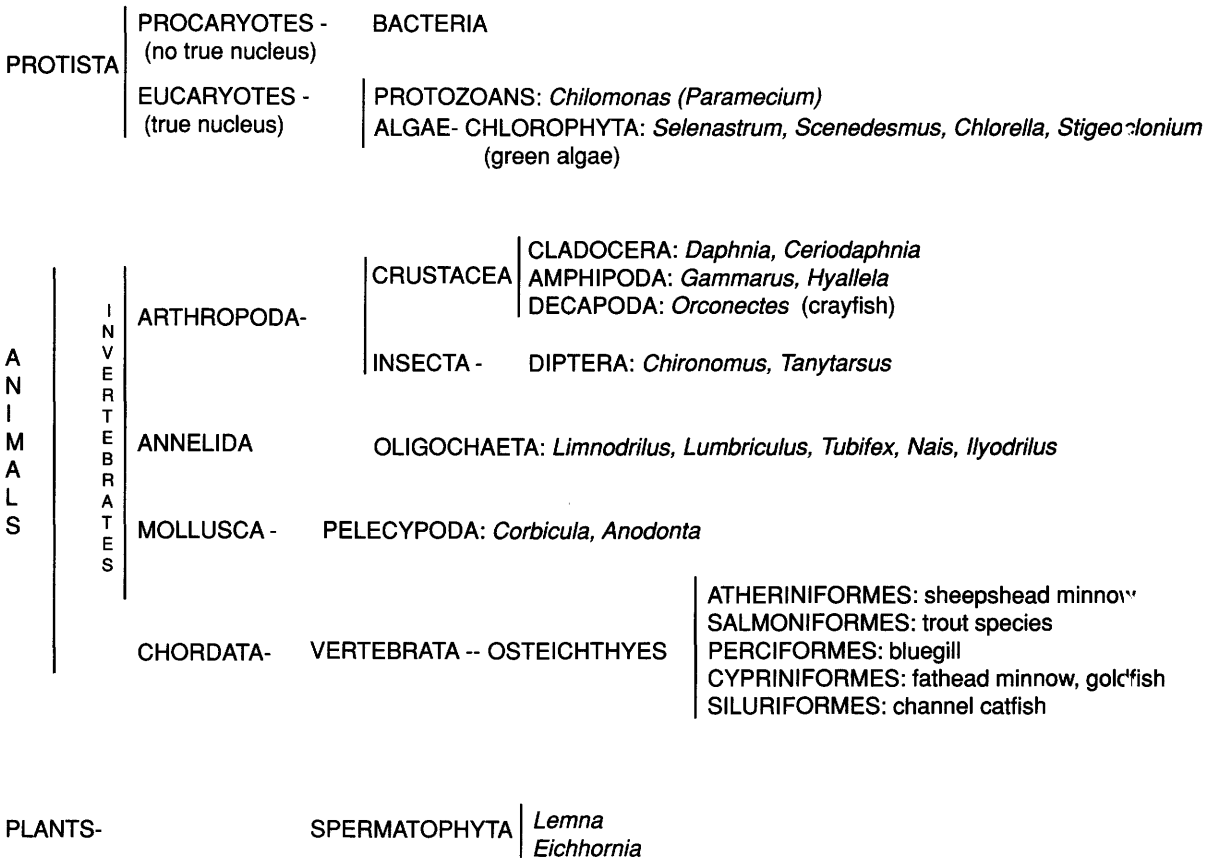

Figure 1. Taxonomic lineages of commonly used freshwater toxicity-test species.

The data for bacteria (table 5) are especially abundant, largely because this methodology has been developing rapidly and advantages are often discussed in support of this development. If table 5 represents an accurate appraisal of bacterial techniques, it is apparent why bacteria are attracting more users. Researchers are almost unanimous in believing that an important reason for using a bacteria test method is simplicity and rapidity (criterion 7). Low cost (criterion 12) also is mentioned frequently. Of greater significance, however, is the frequency with which criteria 1 and 2 (sensitivity and representativeness) were given as advantages of bacteria tests. Investigations that compare the sensitivities of bacteria tests with those of eucaryotic species almost invariably show inferior sensitivity of bacteria. Nevertheless, many authors reported bacterial sensitivities for specific applications that are comparable to, or better than, those of other organisms.

The frequent consideration of sensitivity in selecting a test species is especially intriguing. Sensitivity was mentioned as an advantage of the species chosen for many studies, regardless of what species it was, in spite of evidence (discussed later in this report) of wide discrepancies among species in their sensitivities to specific substances. The apparent contradictions illustrate that sensitivity evaluations should be cast in terms of the toxic agents in question and that sensitivity should be compared with that of other species.

Daphnia magna, for example, has been shown to be one of the most sensitive of common test species for most metals. However, it is not very sensitive to organic compounds, and its sensitivity varies considerably among different classes of organics. Whatever the toxicant, the sensitivity of *Daphnia magna* is based largely on comparison with sensitivities of other species to the same toxicant. The outcome of this comparison is clearly dependent on the species with which *Daphnia* is compared.

The lessons from the sensitivity data in tables 5–10, therefore, are that (1) sensitivity is toxicant dependent and (2) sensitivity is usually assessed by comparing it with the sensitivity of other species rather than by comparing it with some absolute scale based on expected toxicant concentrations in nature. Every species listed in table 3 has been selected as a toxicity-test organism largely because its sensitivity was judged better than that of many other species, at least for some toxicants. Thus, the large number of favorable marks for criterion 1 in tables 5–10 should not be interpreted as an indication of good overall sensitivity to different types of toxicants or of good sensitivity to any

Table 4. Characteristics of flora and fauna used in toxicity tests

Taxonomic group	Characteristics pertinent to use as test species
BACTERIA	Microscopic, unicellular, anatomically simple (no true nucleus or nuclear membrane; no mitotic division). Biochemistry of luminescence or other endpoints is similar to cytochrome-linked respiratory chain common to other organisms. Luminescence requires much energy; hence it is likely to be responsive to toxicants.
PROTOZOANS	Unicellular, but cellular organization is complete, like in multicellular organisms. Cilia or flagella provide mobility, but small size makes protozoans subject to transport by currents or wave action. Chilomonas paramecium, most common test species, injests no particulate food; utilizes dissolved organic matter to synthesize protoplasmic substance.
ALGAE	Commonly used as indicators of water quality (Rawson, 1956; Palmer, 1969). Important ecological niche as primary producers at base of food web. Utilize dissolved substances, thus not affected by toxicants in sediments, except to extent that such pollutants are desorbed into water. Most frequently used test species is single-celled green algae (Chlorophyta). Selenastrum capricornutum, easy to culture, identify, and quantify, is among most commonly used of all test species.
MACROPHYTES	Larger plants, rooted or free floating. Lemna and Eichhornia are both free floating, often in dense populations; can be nuisances by clogging waterways or causing oxygen depletions upon decay. Can also be beneficial as food and shelter for other organisms, and for contributions to photosynthesis and element cycling. Limited toxicological data available (Bowmer, 1986).
NEMATODES	Extremely abundant and widely distributed in all kinds of aquatic systems. Not widely used as test species; poor sensitivity to most toxic agents.
OLIGOCHAETES	Aquatic counterparts to terrestrial earthworms. Many test species have been used, but none widely used. Relative to arthropods, tend to be more tolerant of pesticides, but less tolerant of toxic metals (Brinkhurst and Cook, 1974). Tubifex tubifex tolerant of unfavorable environmental conditions; hence usually considered a pollution indicator.
CLADOCERANS	Extremely common in freshwater systems. Filter feeders. Tend to be more sensitive to metals than to organics. Life cycle includes instars, separated by molts. Daphnia magna most commonly used of all test species. Ceriodaphnia reticulata distinguished from other cladocerans by small size, short life cycle, and common occurrence in a variety of freshwater habitats.
AMPHIPODS	Gammarus species among most commonly used test organisms, especially for sediment-toxicity tests. Life cycle includes instars, separated by molts.
DECAPODS (Crayfish)	Widely distributed, especially in Southeastern United States. Life cycle includes instar stages, separated by molts. During molts, animals are more sensitive to toxicants (Hobbs and Hall, 1974). Orconectes immunis, an active burrower, inhabits sluggish streams and ponds.
INSECTS	Extremely adaptive to all kinds of environments. Great diversity reflects environmental conditions; hence useful bioindicators. Chironomidae is one of largest families--widely distributed and often extremely abundant (up to 50,000 per square meter). Difficult to identify Chironomid species.
MOLLUSKS	Extreme economic importance, both beneficial and detrimental. Filter-feeding bivalves (clams and mollusks) most common test species. Corbicula larval stages are ciliated and free swimming, unlike most other bivalve species. Corbicula can exploit nearly any type of substrate.
FISH	As the only vertebrates commonly used in toxicity tests, fish represent higher trophic levels than other test species. Eggs or early life stages usually more sensitive to toxicants than adults. Contain high lipid concentrations [up to 15 percent of total body weight (Niimi, 1983)]; hence hydrophobic substances, primarly organics, readily accumulate in fish tissue (Chiou, 1985). Extreme mobility often allows escape from toxic sources in natural systems. Pimephales promelas used extensively as toxicity test species, frequently as basis for setting maximum tolerance limits.

References, for more information:

Ward and Whipple, 1959 (invertebrates, macrophytes)
Meglitsch, 1967 (invertebrates)
Prescott, 1970 (algae)
Brinkhurst and Cook, 1974 (oligochaetes)
Mitchell, 1974 (bacteria)
Pennak, 1978 (invertebrates)
Brock, 1979 (bacteria)
Anderson, 1980 (chironomids)
Arthur, 1980 (amphipods)
Benfield and Buikema, 1980 (invertebrates)
Bitton, 1982 (bacteria)
Bone and Marshall, 1982 (fish)
American Public Health Association and others, 1985
Hobbs and Hall, 1974 (crayfish)
Fuller, 1974 (bivalves)
Roback, 1974 (insects)
Rheinheimer, 1974 (bacteria)

particular toxicant at naturally occurring or even maximum allowable concentrations.

Acute Sensitivities of Test Species

Sensitivity is the primary factor that determines the usefulness of a test species (table 2). However, it is not a simple matter to select the most sensitive indicator organism for every test situation. It is especially difficult for ambient tests in which the test substance may contain several toxic agents. Not only is sensitivity dependent on the toxic agent, but there is little information available on which to base evaluations of the relative sensitivities of species to specific agents. This is especially true for chronic tests because of the wide variety of monitoring procedures and endpoints.

Some data have been compiled in tables 11–19 to compare acute sensitivities of different species to certain

Table 5. Evaluation of bacteria species commonly used in toxicity tests with respect to selection criteria listed in table 2

[Data are from published studies in which indicated species were used or discussed. Ratings with respect to numbered criteria are based on statements by authors: "+," advantage of this species over other commonly used species; "−," disadvantage of this species; "±," advantageous in some cases, disadvantageous in others (for example, species may be sensitive to some toxicants and insensitive to others). Absence of symbol indicates that criterion was not mentioned.

Toxicant codes: "var," various; "O," organics, in general; "M," metals; "E," effluents; "N," natural sediments or water.

In all cases, contaminant effects are detected by measuring changes in luminescence or other metabolic functions]

Species	Toxicant(s)	Criterion number												Reference
		1	2	3	4	5	6	7	8	9	10	11	12	
Bacteria (var. species)		+				+		+					+	Greene & others, 1985
Bacteria (var. species)	var		+					+					+	Berkowitz, 1979
Bacteria (var. species)	var	+	+					+						Bulich, 1979
Photobacterium phosphoreum		+				-		+						Dutka & Kwan, 1981
Photobacterium fischeri	O	+	+					+						Lebsack & others, 1981
Photobacterium phosphoreum	O	+	+			+		+						Curtis & others, 1982
Photobacterium phosphoreum	M,O,E	±				+		+					+	Qureshi & others, 1982
Bacteria (var. species)	var	+	+		+	-		+	+				+	Bitton, 1982
Photobacterium phosphoreum	M,O	+	+			+		+			+			De Zwart & Slooff, 1983
Photobacterium phosphoreum	M,O	-				-		+					+	McFeters & others, 1983
Photobacterium phosphoreum	O	+	+		+			+					+	Ribo & Kaiser, 1983
Photobacterium phosphoreum	var	+				+		+						Vasseur & others, 1984
Photobacterium phosphoreum	var	+	+			+		+					+	Coleman & Qureshi, 1985
Spirillum voluntans	var	+	+			+		+					+	Coleman & Qureshi, 1985
Photobacterium phosphoreum	N				+				+	+			+	Schiewe & others, 1985
Pseudomonas putida	M,O	+		+	+				+	+			+	Slabbert, 1986

Table 6. Evaluation of algae species commonly used in toxicity tests with respect to selection criteria listed in table 2

[Data are from published studies in which indicated species were used or discussed. Ratings with respect to numbered criteria are based on statements by authors: "+," advantage of this species over other commonly used species. Absence of symbol indicates that criterion was not mentioned.

Toxicant codes: "var," various; "M," metals; "PC," petroleum or coal tar derivatives; "OH," organic herbicides; "N," natural sediments or water; "O," organics, in general.

In all cases, contaminant effects are detected by measuring changes in growth, productivity, or other metabolic functions]

Species	Toxicant(s)	Criterion number												Reference
		1	2	3	4	5	6	7	8	9	10	11	12	
Selenastrum capricornutum (and other species)	var			+			+							Payne & Hall, 1979
Selenastrum capricornutum	M									+				Christensen & others, 1979
Selenastrum capricornutum	PC	+												Giddings & others, 1983
Scenedesmus quadricauda	OH	+												Aly & others, 1984
Selenastrum capricornutum	N	+												Eloranta & Halttunen-Keyrilainen, 1984
Selenastrum capricornutum	O			+						+	+			Adams, Goulding, & Dobbs, 1985
Stigeoclonium tenue	N						+							De Vries & Hotting, 1985
Selenastrum capricornutum	OH	+												Meyerhoff & others, 1985
Selenastrum capricornutum (and other species)	O	+												Gaur & Kumar, 1981

organic compounds and metals in water. Despite the fact that methods and test conditions vary, single-species acute toxicity tests provide data in a standard format for specific toxic agents. Other kinds of tests do not produce this kind of comparable information. Therefore, results of chronic tests, or results from studies that were done with ambient substances or variable mixtures of substances (such as leachates or effluents), could not be included.

The endpoint for all animal species was mortality, and the results were reported as LC50, in milligrams per liter. There was some variation in exposure time, but in most cases it was 96 hours for fish, 48 hours for cladocer-

Table 7. Evaluation of *Daphnia magna* with respect to selection criteria listed in table 2

[Data are from published studies in which *Daphnia magna* was used or discussed. Ratings with respect to numbered criteria are based on statements by authors: "+," advantage of this species over other commonly used species; "−," disadvantage of this species. Absence of symbol indicates that criterion was not mentioned.

Test type codes: "A," acute; "C," chronic.

Toxicant codes: "var," various; "O," organics, in general; "M," metals; "OP," organic pesticides; "N," natural sediments or water]

Test type	Toxicant(s)	Criterion number 1	2	3	4	5	6	7	8	9	10	11	12	Reference
AC	var	+			+		+		+					Adema, 1978
A	var	+	+											Kenaga, 1978
AC	var	+			+	+			+					Ten Berge, 1978
A	O	+												Dill & others, 1982
A	var		+							+	+			LeBlanc, 1980
AC	M,OP				−			−					−	Nebeker, 1982
A	O					−		−						Barera & Adams, 1983
A	M,O			+	+				+		−			Berglind and Dave, 1984
AC	var	+			−		−		−	+			−	Mount & Norberg, 1984
ACS	N	+			+			+					+	Nebeker & others, 1984
A	M,O	+			−	−								Lewis & Weber, 1985

Table 8. Evaluation of Cladocerans, excluding *Daphnia magna*, commonly used in toxicity tests with respect to selection criteria listed in table 2

[*D.*, *Daphnia*; *C.*, *Ceriodaphnia*. Data are from published studies in which indicated species were used or discussed. Ratings with respect to numbered criteria are based on statements by authors: "+," advantage of this species over other commonly used species; "−," disadvantage of this species. Absence of symbol indicates that criterion was not mentioned.

Test type codes: "A," acute; "C," chronic.

Toxicant codes: "var," various; "O," organics, in general; "OP," organic pesticides; "M," metals]

Species	Test type	Toxicant(s)	Criterion number 1	2	3	4	5	6	7	8	9	10	11	12	Reference
D. pulex	A	var	+	+											Kenaga, 1978
var	A	var	+			+	+	+							Leeuwangh, 1978
var	A	chromium			+	+	−	+			+	+		+	Lee & Buikema, 1979
Daphnia spp.	AC	var	+			−			−					−	Buikema & others, 1980
D. pulicaria	A	O	−												DeGraeve & others, 1980
C. reticulata	C	var	+			+			+					+	Mount & Norberg, 1984
D. laevis	A	OP						+							Foran & others, 1985
D. pulex	A	M,O	+			−	−								Lewis & Weber, 1985
C. reticulata	A	M,O	+												Elnabarawy & others, 1986

ans, and either 48 or 96 hours for other invertebrates. For bacteria, the endpoint was 50-percent reduction in luminescence, and for algae, it was 50-percent reduction in growth or production. Although different from mortality, these standardized endpoints for bacteria and algae are well established and may be compared with animal LC50's for the purpose of comparing sensitivities.

The data in tables 11–17 indicate some patterns of sensitivity among different species, and they also raise some questions. Bacteria are apparently sensitive to organics under some conditions, but they are much less useful for testing metal toxicity. Mercury is a possible exception; it produces responses in *Photobacterium phosphoreum* at relatively low concentrations (table 18). Cladocerans are

Table 9. Evaluation of *Pimephales promelas* (fathead minnows) with respect to selection criteria listed in table 2

[Data are from published studies in which *Pimephales promelas* was used or discussed. Ratings with respect to numbered criteria are based on statements by authors: "+" advantage of this species over other commonly used species. Absence of symbol indicates that criterion was not mentioned.

Test type codes: "A," acute; "C," chronic.

Toxicant codes: "M," metals; "O," organics, in general; "var," various; "E," effluents]

Test type	Toxicant(s)	Criterion number												Reference
		1	2	3	4	5	6	7	8	9	10	11	12	
AC	M,O			+	+									Adelman & Smith, 1976
C	var	+				+							+	McKim, 1977
A	O	+								+	+			Spehar & others, 1982
A	E	+	+				+							Keefe & others, 1983
C	toluene	+			+		+			+				Devlin & others, 1985
C	E	+			+		+	+		+			+	Norberg & Mount, 1985b

Table 10. Evaluation of fish species, excluding *Pimephales promelas*, commonly used in toxicity tests with respect to selection criteria listed in table 2

[Data are from published studies in which indicated species were used or discussed. Ratings with respect to numbered criteria are based on statements by authors: "+," advantage of this species over other commonly used species. Absence of symbol indicates that criterion was not mentioned.

Test type codes: "A," acute; "C," chronic.

Toxicant codes: "var," various; "M," metals; "O," organics, in general; "E," effluents]

Fish	Test type	Toxicant(s)	Criterion number											Reference
			1	2	3	4	5	6	7	8	9	10	11	12	
Rainbow trout	A	var	+	+											Kenaga, 1978
Salmonids (various)	A	M						+							Davies & Woodling, 1980
Rainbow trout	A	phenolics	+												DeGraeve and others, 1980
Bluegill	C	var			+						+	+			van der Schalie, 1980
Rainbow trout	A	O	+												Dill & others, 1982
Bluegill	A	E	+	+				+							Keefe & others, 1983

generally among the most sensitive species to metals. Some of the less commonly used invertebrates, such as *Corbicula* species and some of the amphipods and oligochaetes, show relatively high sensitivity to some substances.

Among the peculiarities shown by the tables are the wide ranges of sensitivities, even within a single species. For example, there was a 100-fold range in sensitivity to benzene by *Photobacterium phosphoreum* in different studies (table 13). A similar range appeared in the sensitivity of *Daphnia magna* to toluene (table 14), and the overall range in sensitivity to toluene was nearly 3,000-fold among just six studies. Fish species generally are considered especially good indicators for organic contaminants, but this contention is not strongly supported by tables 11–14. None of the tables reveals a clearly superior species in terms of its sensitivity to that substance.

Another disturbing aspect of sensitivity revealed by tables 11–19 is that even the best sensitivities shown are not indicative of truly useful bioindicators. The lowest concentrations shown for each of the toxicants represented are higher than any that would be encountered in most aquatic systems, except for highly contaminated waters. An illustration of this point is shown in figure 2. The LC50 values for copper, cadmium, zinc, mercury, and lead are taken from tables 15–19. Thus, they represent lethal concentrations in various acute tests, using different test species. The actual concentration data, shown by the histograms, are the maximum and median total concentrations of the same metals at some sites around the country that have been monitored by the U.S. Geological Survey (USGS) for several years. These sites are subject to inputs from various industrial, agricultural, or municipal sources and may be

Table 11. Acute toxicities of phenol to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Daphnia magna	48 h	6.6	Keen and Baillod, 1985
Daphnia magna	48 h	12	LeBlanc, 1980
Photobacterium phosphoreum	5 m	22	Qureshi and others, 1982
Photobacterium phosphoreum	5 m	25	Lebsack and others, 1981
Photobacterium phosphoreum	5 m	25	Bulich and others, 1981
Photobacterium phosphoreum	5 m	26	Chang and others, 1981
Photobacterium phosphoreum	5 m	28	Dutka and others, 1983
Daphnia magna	48 h	30	Bobra and others, 1983
Photobacterium phosphoreum	15 m	34	Dutka and Kwan, 1981
Photobacterium phosphoreum	5 m	39.5	McFeters and others, 1983
Photobacterium phosphoreum	5 m	40.2	Curtis and others, 1982
Pimephales promelas	96 h	67.5	DeGraeve and others, 1980
Daphnia pulicaria	48 h	>109	DeGraeve and others, 1980
Pseudomonas putida	6 h	244	Slabbert, 1986

expected to contain higher metal concentrations than most natural waters. Nevertheless, nearly all the maximum concentrations of cadmium, mercury, and lead reported from these sites are lower than the LC50 values shown, and many are lower by a factor of 10 or more. Even for copper and zinc, the natural concentrations are lower than most LC50 values shown. Although sublethal effects or lower percentage mortality may occur at lower concentrations than the LC50 values shown in figure 2, these data cast serious doubt that most current test procedures would have adequate sensitivity to reveal long-term toxicant conditions in ambient waters.

Figure 2 also compares LC50 data with water-quality criteria established by the U.S. Environmental Protection Agency (1986). Acute criteria are concentrations likely to be detrimental to aquatic life if exceeded for a 1-hour period at least once during 3 years, on the average. Chronic criteria are concentrations likely to be detrimental to aquatic life if exceeded for a 4-day period at least once during 3 years, on the average. The acute criteria are lower than nearly all of the LC50 values given. The chronic criteria are even further below the detection ranges for the toxicity tests; they are less than LC50 values for all methods shown except zinc.

Overview of Test-Species Selection

The preceding discussion of test species and criteria for their selection does not lead to clear choices of species to use for a large-scale water-quality-assessment program or for any particular study within such a program. It does include information that should be considered before selecting species. The selections themselves would necessarily depend on conditions of the test and application requirements, which would vary from study to study.

Certain criteria from table 2 are especially important for selection of procedures to investigate potential ambient toxicity problems over broad geographical areas. These include amenability to laboratory culture (criterion 4), reproducibility of results (5), simplicity of test (7), availability of background information (9), documented methodology (10), and low cost (12). All of these factors relate to the practicality of using the species in a standard fashion in a variety of different test waters, and (or) to the interpretability and comparability of data obtained in this way. Presumably, therefore, these criteria would move up on the scale of relative importance in selecting test species for a national program.

Sensitivity (criterion 1) remains a very important criterion for test-species selection in large-scale ambient toxicity assessments. If the test is so insensitive that toxicity detection is not likely even with heavily contaminated samples, then it is of little use as a biomonitoring tool, regardless of its other attributes. Representativeness (criterion 2) also is a very important selection criterion if there is any expectation of analyzing the biological implications of contaminant occurrences.

The test species selected should suit the stated purpose of the test. For example, if rainbow trout is the key species in the study area that might be affected by a contaminant input, then rainbow trout would be the logical choice as a test species. Most selections are likely to be far less obvious, but consideration of the advantages and disadvantages of different test species should improve the likelihood of success.

In general, any toxicity-testing procedure that uses only one test species is not appropriate for determining the effects of the broad array of contaminants that may be present over a large geographical area. The current status of toxicity-test methodology and information about sensitivities of test species indicate that no single-species test has the general applicability and uniformly high sensitivity required for ambient toxicity testing on a regional or national scale. There are greater possibilities if more than one species can be used; more discussion of this option follows.

Acute and Chronic Tests

One of the most important methodology decisions to be made in designing a single-species test is whether testing

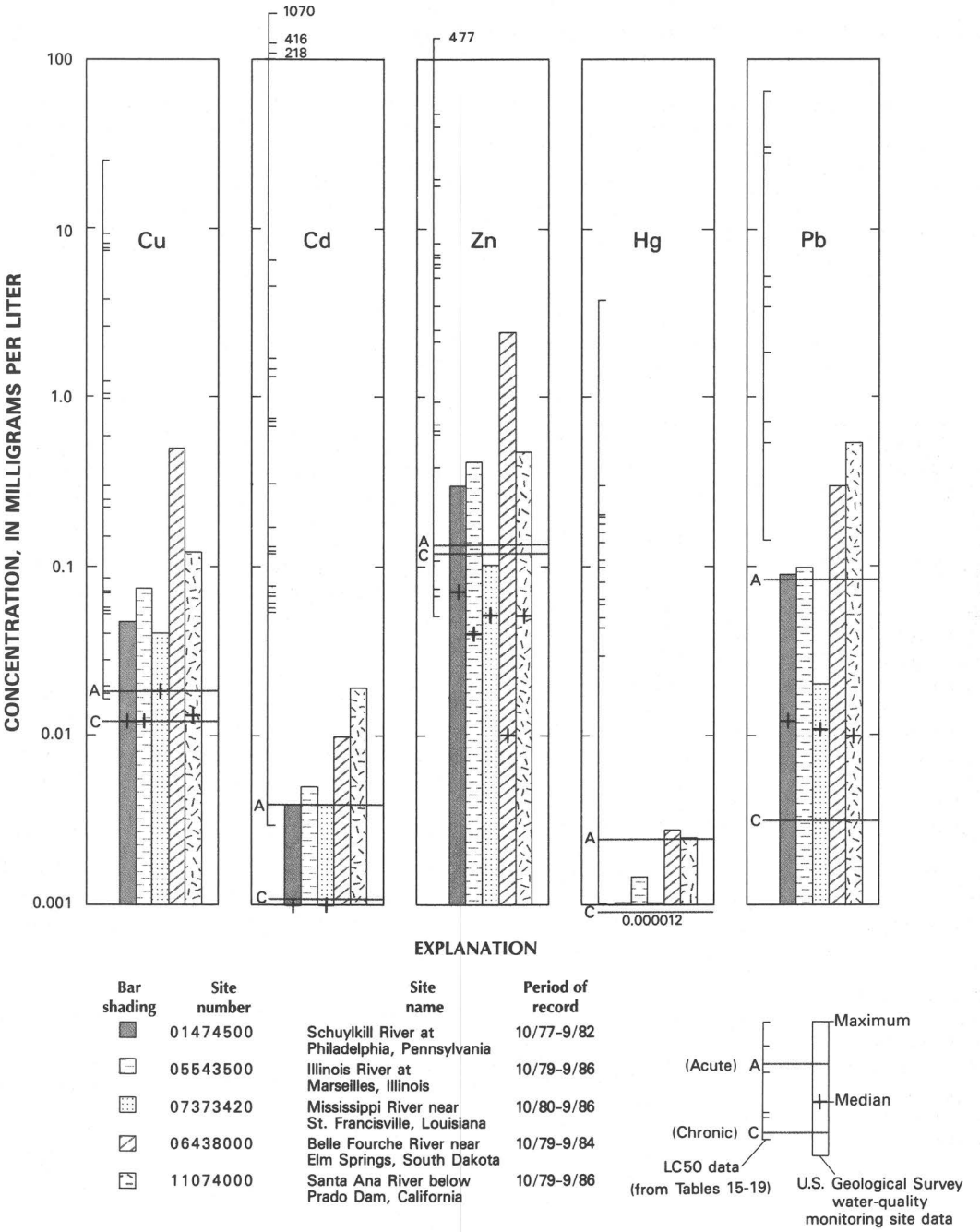

Figure 2. LC50 (50-percent lethal concentration) data from tables 15–19 compared with maximum and median concentration data for copper, cadmium, zinc, mercury, and lead in water from five selected U.S. Geological Survey water-quality monitoring sites and water-quality criteria.

should be done by acute lethality tests or chronic exposure tests.

In an acute test, the test organisms are exposed to relatively high concentrations of the contaminant, and the test is concluded in a short time (usually a few days). The common endpoint of such tests is mortality, measured as LC50, which is the minimum concentration that causes 50-percent mortality of test organisms during a specified time period (usually 48 or 96 hours). The test generally is done with a single species in small enclosures in a laboratory. In the simplest method, the "static" acute test, the medium and its toxicant content are not changed during the experiment. Alternatives to this method are "static-renewal," "flow-through," and "continuous-flow" tests, in

Table 12. Acute toxicities of pentachlorophenol to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Ictalurus punctatus (catfish)	96 h	0.053	Phipps and Holcombe, 1985
Photobacterium phosphoreum	5 m	.08	Curtis and others, 1982
Salmo gairdneri	96 h	.093	McKim and others, 1987
Nais communis	96 h	.11	Chapman and Mitchell, 1986
Salmo gairdneri	96 h	.115	Thurston and others, 1985
Lepomis macrochirus	96 h	.14	Phipps and Holcombe, 1985
Daphnia magna	48 h	.143	Mount and Norberg, 1984
Daphnia magna	48 h	.145	Thurston and others, 1985
Carassius auratus (goldfish)	96 h	.16	Phipps and Holcombe, 1985
Ceriodaphnia reticulata	48 h	.164	Mount and Norberg, 1984
Carassius auratus	96 h	.190	Adelman and others, 1976
Salmo trutta	24 h	.2	Hattula and others, 1981
Lepomis macrochirus	96 h	.202	Thurston and others, 1985
Pimephales promelas	96 h	.203	Adelman and others, 1976
Daphnia pulex	48 h	.246	Mount and Norberg, 1984
Pimephales promelas	96 h	.25	Phipps and Holcombe, 1985
Carassius auratus	96 h	.264	Thurston and others, 1985
Pimephales promelas	96 h	.266	Thurston and others, 1985
Ilyodrilus frantzi	96 h	.31	Chapman and Mitchell, 1986
Daphnia magna	48 h	.33	Lewis and Weber, 1985
Limnodrilus hoffmeisteri	96 h	.33	Chapman and others, 1982a
Tubifex tubifex	96 h	.38	Chapman and others, 1982a
Daphnia pulex	48 h	.39	Lewis and Weber, 1985
Photobacterium phosphoreum	5 m	.5	Bulich and others, 1981
Daphnia magna	48 h	.68	LeBlanc, 1980
Photobacterium phosphoreum	15 m	.76	De Zwart and Slooff, 1983
Photobacterium phosphoreum	5 m	.94	De Zwart and Slooff, 1983
Tanytarsus dissimilis	48 h	25.2	Thurston and others, 1985
Pseudomonas fluorescens	1 h	29.2	Trevors and others, 1982
Orconectes immunis	96 h	183	Thurston and others, 1985

which the medium and its toxicant load are continually or periodically replenished as spent medium flows from the test enclosure.

The LC50 concept is relatively simple and is widely used. Lethality is an easily monitored endpoint, and LC50 provides a convenient, standardized, and unambiguous format for reporting toxicities. However, it has been argued that LC50 is an arbitrary and meaningless standard that is irrelevant to the natural environment (White and Champ, 1984). Certainly, it is an indicator of the toxicity of a particular substance to a particular organism under the conditions of the test; however, it is not at all clear what that

Table 13. Acute toxicities of benzene to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Photobacterium phosphoreum	5 m	2.0	Bulich and others, 1981
Photobacterium phosphoreum	5 m	4.11	McFeters and others, 1983
Daphnia magna	48 h	31.3	Bobra and others, 1983
Photobacterium phosphoreum	5 m	200	Chang and others, 1981
Daphnia magna	48 h	200	LeBlanc, 1980
Photobacterium phosphoreum	5 m	214	De Zwart and Slooff, 1983
Photobacterium phosphoreum	15 m	238	De Zwart and Slooff, 1983

means in terms of overall toxicity of the substance in the natural environment. Some reports show a good correlation between LC50 and measures of natural toxicant effects (Mount and others, 1984; Giddings and Franco, 1985), whereas others show a poor relation (Rodgers and others, 1980; Kimball and Levin, 1985). Numerous authors have cautioned against assumptions of community toxicity based on measurements with a single species or process (Cairns, 1983; Dutka and others, 1983; Blanck, 1984; Bowmer, 1986).

Table 14. Acute toxicities of toluene to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Daphnia magna	48 h	11.5	Bobra and others, 1983
Pimephales promelas	96 h	26	Devlin and others, 1982
Photobacterium phosphoreum	5 m	50	Chang and others, 1981
Pimephales promelas (embryos)	96 h	63	Devlin and others, 1982
Dapnia magna	48 h	310	LeBlanc, 1980
Photobacterium phosphoreum	5 m	33,833	McFeters and others, 1983

Table 15. Acute toxicities of copper to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Ceriodaphnia reticulata	48 h	0.017	Mount and Norberg, 1984
Daphnia magna	48 h	.02	Qureshi and others, 1982
Corbicula manilensis (veliger larvae)	24 h	.028	Harrison and others, 1984
Corbicula fluminea	96 h	.04	Rodgers and others, 1980
Daphnia pulex	48 h	.053	Mount and Norberg, 1984
Daphnia magna	48 h	.054	Mount and Norberg, 1984
Selenastrum capricornutum		.054	Turbak and others, 1986
Chlorella stigmatophora		.07	Christensen and others, 1979
Selenastrum capricornutum		.07	Bartlett and others, 1974
Selenastrum capricornutum		.085	Christensen and others, 1979
Lumbriculus variegatus	96 h	.15	Bailey and Liu, 1980
Lumbriculus variegatus	48 h	.23	Bailey and Liu, 1980
Salmo gairdneri	96 h	.25	Qureshi and others, 1982
Chironomus tentans (1st instar)	96 h	.30	Nebeker, Cairns, and Wise, 1984
Corbicula fluminea	24 h	.59	Rodgers and others, 1980
Lepomis macrochirus	96 h	1.0	Thompson and others, 1980
Pseudomonas putida (bact.)	6 h	1.05	Slabbert, 1986
Crangonyx pseudogracilis (amphipod)	96 h	1.29	Martin and Holdich, 1986
Corbicula manilensis (adult)	96 h	2.6	Harrison and others, 1984
Photobacterium phosphoreum	15 m	3.8	Dutka and Kwan, 1981
Spirillum voluntans	5 m	7.4	Qureshi and others, 1982
Photobacterium phosphoreum	5 m	7.4	Qureshi and others, 1982
Photobacterium phosphoreum	5 m	8.0	Bulich and others, 1981
Asellus aquaticus (amphipod)	96 h	9.21	Martin and Holdich, 1986
Photobacterium phosphoreum	5 m	25	McFeters and others, 1983

Table 16. Acute toxicities of cadmium to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Salmo gairdneri	96 h	0.003	Phipps and Holcombe, 1985
Daphnia magna	48 h	.053	Lewis and Weber, 1985
Selenastrum capricornutum		.057	Turbak and others, 1986
Selenastrum capricornutum		.06	Bartlett and others, 1974
Ceriodaphnia reticulata	48 h	.066	Mount and Norberg, 1984
Daphnia pulex	48 h	.068	Mount and Norberg, 1984
Lumbriculus variegatus	96 h	.074	Bailey and Liu, 1980
Daphnia magna	48 h	.118	Mount and Norberg, 1984
Lumbriculus variegatus	48 h	.12	Bailey and Liu, 1980
Gammarus pulex	96 h	.12	Wright and Frain, 1981
Limnodrilus hoffmeisteri	96 h	.17	Chapman and others, 1982a
Tubifex tubifex	96 h	.32	Chapman and others, 1982a
Gammarus pulex	48 h	.68	Wright and Frain, 1981
Pseudomonas putida (bact.)	6 h	.72	Slabbert, 1986
Carassius auratus	96 h	.748	Phipps and Holcombe, 1985
Asellus aquaticus (amphipod)	96 h	1.32	Martin and Holdich, 1986
Pimephales promelas	96 h	1.5	Phipps and Holcombe, 1985
Crangonyx pseudogracilis (amphipod)	96 h	1.70	Martin and Holdich, 1986
Ictalurus punctatus	96 h	4.48	Phipps and Holcombe, 1985
Lepomis macrochirus	96 h	6.47	Phipps and Holcombe, 1985
Photobacterium phosphoreum	15 m	218	De Zwart and Slooff, 1983
Photobacterium phosphoreum	5 m	416	McFeters and others, 1983
Photobacterium phosphoreum	5 m	1,070	De Zwart and Slooff, 1983

In a chronic test, the organisms are exposed to nonlethal concentrations over a relatively long period of time. Long-term exposure may produce some mortality, but the endpoint of the experiment is some sublethal response such as decrease in growth rate, reduction of reproductive capacity, interference with mobility, or anatomical change. The results yield information about "effective" concentrations of the toxic agent rather than the lethal concentrations determined by acute tests. Sublethal responses might not occur during a short-term test. For example, Winner (1981) found that copper and zinc affect the longevity of *Daphnia magna*, but those effects did not appear until some 50–70 days after initiation of the experiment. Many chronic test endpoints can be determined only by monitoring the complete life cycle of the organism (Goodman and others, 1982). Such "life cycle" tests must be continued for at least as long as one reproductive cycle. Chronic tests are potentially more informative than acute tests, not only because they avoid the problems of short, unrealistic exposures (Eaton, 1973), but also because more data are generated, allowing more rigorous statistical analysis (Brown, 1973).

A number of recent studies of the effects of toxicants on a variety of animal species have emphasized the improved sensitivity of chronic tests over acute tests (Eaton, 1973; Sprague, 1976; Winner and Farrell, 1976; Winner, 1981, Snarski and Olson, 1982; Chapman and Brinkhurst, 1984; Hermens and others, 1984; Chapman and others, 1985; Norberg and Mount, 1985b). Sublethal responses may be observed at toxicant concentrations considerably lower than those that produce mortality of half of the

Table 17. Acute toxicities of zinc to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; d, days; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Selenastrum capricornutum		0.051	Turbak and others, 1986
Daphnia magna	48 h	.068	Mount and Norberg, 1984
Ceriodaphnia reticulata	48 h	.076	Mount and Norberg, 1984
Daphnia pulex	48 h	.107	Mount and Norberg, 1984
Salmo gairdneri	14 d	.41	Nehring and Goettl, 1974
Salmo trutta	14 d	.64	Nehring and Goettl, 1974
Salmo clarki	14 d	.67	Nehring and Goettl, 1974
Selenastrum capricornutum		.7	Bartlett and others, 1974
Salvelinus fontinalis	14 d	.96	Nehring and Goettl, 1974
Salmo gairdneri	96 h	2.2	Qureshi and others, 1982
Photobacterium phosphoreum	5 m	2.5	Bulich and others, 1981
Photobacterium phosphoreum	15 m	3.5	Dutka and Kwan, 1981
Daphnia magna	48 h	5.1	Qureshi and others, 1982
Corbicula fluminea	96 h	6.04	Rodgers and others, 1980
Lumbriculus variegatus	96 h	6.3	Bailey and Liu, 1980
Pseudomonas putida (bact.)	6 h	7.15	Slabbert, 1986
Spirillum voluntans	5 m	7.2	Qureshi and others, 1982
Lumbriculus variegatus	48 h	8.1	Bailey and Liu, 1980
Asellus aquaticus (amphipod)	96 h	18.2	Martin and Holdich, 1986
Crangonyx pseudogracilis (amphipod)	96 h	19.8	Martin and Holdich, 1986
Corbicula fluminea	24 h	>40	Rodgers and others, 1980
Photobacterium phosphoreum	5 m	49	Qureshi and others, 1982
Photobacterium phosphoreum	5 m	477	McFeters and others, 1983

population. However, this advantage of chronic testing must be weighed against the disadvantage that sublethal responses are often subtle and difficult to monitor. Inconsistent responses among different individuals of a population is a common problem in chronic tests (Geiger and Buikema, 1981). Furthermore, chronic tests may require complex experimental setups and long-term culturing of organisms.

In part because of their sensitivity, chronic tests may produce more meaningful results than acute tests. Factors such as growth, fecundity, and feeding habits may be more significant indicators of contaminant impacts than lethality at relatively high concentrations. This argument is based on the presumption that environmental contamination, even in extreme cases of pollution, generally will be lower than acute lethal levels but will present the biota with long-term, low-level exposure. Hence, the effects are likely to be sublethal, but still may severely affect the community.

Table 18. Acute toxicities of mercury to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Crangonyx pseudogracilis (amphipod)	96 h	0.001	Martin and Holdich, 1986
Daphnia magna	48 h	.03	Qureshi and others, 1982
Photobacterium phosphoreum	15 m	.044	De Zwart and Slooff, 1983
Photobacterium phosphoreum	5 m	.051	De Zwart and Slooff, 1983
Photobacterium phosphoreum	5 m	.06	McFeters and others, 1983
Photobacterium phosphoreum	5 m	.065	Bulich and others, 1981
Photobacterium phosphoreum	5 m	.08	Qureshi and others, 1982
Lumbriculus variegatus	96 h	.10	Bailey and Liu, 1980
Lumbriculus variegatus	48 h	.11	Bailey and Liu, 1980
Tubifex tubifex	96 h	.14	Chapman and others, 1982a
Nais communis	96 h	.16	Chapman and Mitchell, 1986
Limnodrilus hoffmeisteri	96 h	.18	Chapman and others, 1982a
Asellus aquaticus (amphipod)	96 h	.20	Martin and Holdich, 1986
Salmo gairdneri	96 h	.21	Qureshi and others, 1982
Ilyodrilus frantzi	96 h	.29	Chapman and Mitchell, 1986
Spirillum voluntans	5 m	3.7	Qureshi and others, 1982

Table 19. Acute toxicities of lead to various test species

[Species listed in decreasing order of reported sensitivity (increasing order of concentrations needed to reach endpoint). When ranges of endpoint concentrations were reported by authors, only median or mean values are reported here. Endpoint for animal species is 50-percent mortality in specified exposure time period unless otherwise indicated. Endpoint for bacteria species is 50-percent reduction in measured activity (usually luminescence or mobility) in specified exposure time period. Endpoint for phytoplankton species is 50-percent reduction in growth rate (usually measured as carbon-14 uptake or oxygen production). h, hours; m, minutes]

Species	Exposure time	Endpoint concentration (milligrams per liter)	Reference
Selenastrum capricornutum		0.14	Christensen and others, 1979
Ceriodaphnia reticulata	48 h	.53	Mount and Norberg, 1984
Chlorella stigmatophora		.70	Christensen and others, 1979
Lumbriculus variegatus	96 h	1.8	Bailey and Liu, 1980
Lumbriculus variegatus	48 h	3.4	Bailey and Liu, 1980
Daphnia magna	48 h	4.4	Mount and Norberg, 1984
Daphnia pulex	48 h	5.1	Mount and Norberg, 1984
Crangonyx pseudogracilis (amphipod)	96 h	27.6	Martin and Holdich, 1986
Photobacterium phosphoreum	15 m	30	Dutka and Kwan, 1981
Asellus aquaticus (amphipod)	96 h	64.1	Martin and Holdich, 1986

Despite the repeated claims of the advantages of the chronic-test approach, the simplicity and precision of acute tests are significant factors in their favor. The continued use of acute, single-species tests is ensured by their applications for determining compliance of effluents with regulatory standards. Macek and others (1978) reported that the consensus among attendees at a 1977 workshop on application of aquatic toxicity methods was that acute lethality tests are the most useful of various types of toxicity tests. This was because they were judged the most practical means for determining relative toxicities of various chemicals, relative sensitivities of different species, and effects of water quality on the toxicity of chemicals. However, chronic-testing methodology has developed considerably since the time of that workshop.

Design of Test Enclosure

Most nonbacterial toxicity tests entail enclosure of test organisms in some variation of a static laboratory aquarium of relatively small, manageable size. When the test organisms are macroscopic in size, static enclosures generally hold just one or a few individuals. The static enclosure has the distinct advantage of experimental control and reproducibility. Extraneous variables that might affect test results are minimized, and responses are relatively easy to measure. On the other hand, it has the disadvantage of presenting the test organisms with a very unnatural habitat. Their responses to stress in such a setting may be different than if they were in their natural environment, surrounded by other species with which they interact.

Various alternatives to static enclosures have been used with increasing frequency in recent years, especially for multispecies tests and for tests in which the principal endpoint is something other than acute lethality. Although the static-enclosure approach remains the most popular, other methods have been encouraged by improvements in toxicity-test methodology, including the development of complex design features and more sensitive measurement techniques. The selection of test-enclosure type depends largely on the test species used and the response endpoints monitored. Some species selections, such as bacteria, leave no choices of test enclosures.

Multispecies Tests

A toxicity test need not be limited to a single species. Multispecies tests have been used with increasing frequency in recent years (Hansen and Tagatz, 1980; Kaushik and others, 1985; Phipps and Holcombe, 1985). Their advantages have been pointed out by Maciorowski and Clarke (1980), Suter (1983), Cairns (1983, 1984, 1985), and Kimball and Levin (1985). The most frequent argument is that relative to a single-species test, the multispecies view offers the researcher a more complete and more realistic picture of probable toxicant effects on the entire community. Although there may be some loss in control of untested variables and standardization of procedures, it is argued that this is compensated for by improved realism, completeness, and even sensitivity (Suter, 1983; Kimball and Levin, 1985). There is also an economical argument in support of multispecies testing. Costs per experiment are likely to be higher than single-species tests, but because the amount and quality of information is enhanced, the cost/benefit ratio can be reduced (Suter, 1983; Perez and Morrison, 1985).

It is important to clarify the meaning of "multispecies testing." Two entirely different approaches may be signified by this term. One is a "microcosm" approach, whereby a number of different species are exposed simultaneously to the same environmental stress. Under these conditions, the test "species" is actually a community of species that can interact with each other. Such tests are conducted with the hope that they mimic the natural environment. Rather than showing how the survival or metabolic activities of only one species is affected, these tests are expected to indicate how the toxicant will affect community characteristics such as succession, diversity, predator-prey relations, or dominant taxa. The "multispecies" approach may also refer to a "battery of tests" in which the toxicity problem is examined by conducting a number of separate single-species tests. A different species is used in each test, and collectively they represent distinct trophic levels and (or) are sensitive to different types of toxicants. For example, a cladoceran species and a fish species might be used in a battery. In addition to representing different trophic levels, the cladoceran is likely to be more sensitive to metals, whereas the fish is more sensitive to organic compounds. The tests may be conducted simultaneously or sequentially (Cairns, 1983).

Microcosm Approach

Considerable success has been reported by authors using laboratory microcosms. Portier (1985) cited evidence from microbial studies to support use of benchtop microcosms as a toxicity-testing tool. Correlation coefficients generally greater than 0.9 were reported between lab and field measurements of a number of microbial population parameters and metabolic activities. Harrass and Taub (1985) described a standardized aquatic microcosm designed to be an especially replicable system. The experimental systems were treated with copper, and responses were compared with responses in untreated microcosms and with reported results from field studies. Responses of the microcosms, with respect to algal/grazer interactions, species shifts in algal communities, and recovery of the systems when the toxicant treatment was terminated, were similar to those generally observed in natural systems. Levy and others (1985) compared the pelagic epilimnion of a

California reservoir with three microcosms containing water from the same reservoir. No toxicants were added, but the effects of water agitation were examined. For 6 weeks, the phytoplankton and zooplankton communities of two of the microcosms were virtually indistinguishable from those of the natural system.

Microcosms designed as "in situ" test enclosures have been used to mimic the natural environment as closely as possible. By suspending translucent enclosures on a vertical line, Marshall and Mellinger (1980) tested the effects of depth on responses of plankton to cadmium addition in Lake Michigan. Depth was indeed found to have an effect. The "limnocorral," a large in situ enclosure placed in lakes to represent the natural pelagic community, was used by Kaushik and others (1985) and Herman and others (1986) to examine the effects of pesticides on plankton. The limnocorral technique was described as "an important tool for assessment of direct and indirect impacts" of toxicants (Kaushik and others, 1985).

Some general disadvantages of microcosm-type multispecies procedures were discussed by Mount (1985) and Slooff (1985). Costs and practical restrictions do not allow multispecies experiments to be fully representative of their simulated natural environments. The resulting generality in test design reduces sensitivity and predictability. Analysis of data from studies in which both single-species and multispecies tests have been done shows little difference in the results or conclusions of the different approaches. This led Slooff (1985) to conclude that "as long as there is no solid evidence that predictions made from single species tests are unreliable, there are no imperative reasons to propose expensive and time-consuming multispecies tests as additional or alternative research tools." Mount (1985) pointed out that if the primary purpose of the toxicological work is to examine the effects on a valuable resource species (for example, a sport fishery), single-species tests are certainly more suitable. He also suggested that the claims of improved realism and sensitivity may be misleading: "...community sensitivity is only an expression of individual species sensitivity...that there are interactions between species in multispecies tests is not a measure of their validity or informative value. In fact, the reverse could well be true!"

Numerous specific microcosm applications have demonstrated weaknesses in the microcosm approach. A three-phase (gaseous-aqueous-sediment) microcosm was used by Adams, Werner, and others (1985) to simulate Lake Powell, Utah-Arizona, and to study effects of and fate of benz(a)anthracene (BA). Results in the microcosm were representative of simultaneous field measurements, but differences in physical conditions caused significant differences in BA behavior. For example, reduced light levels substantially diminished the rate of photooxidation. Open microcosms were used by Selby and others (1985) to assess the effects of cadmium on a stream community. Because of the possibility of community changes unrelated to the toxicant, the method was not recommended for use as a screening tool. Woltering (1983) found that responses to toxicants in laboratory ecosystem studies were highly dependent on ecological factors such as predator-prey fluctuations, competition, energy input, and habitat availability. Predation can be an especially important controlling factor, and must be at least partly restricted in most microcosm studies (Harrass and Taub, 1985). Aging (nutrient depletion) of the microcosm can also influence toxicant effects on test organisms (Kindig and others, 1983).

At the 1977 workshop on estimating the hazard of chemicals to aquatic life, where attendees evaluated various toxicity-test techniques (Macek and others, 1978), the microcosm approach was rated rather low in overall utility. It was considered inferior to most other techniques in ecological significance, scientific and legal defensibility, and simplicity and cost. Two of the participants in that workshop (Brungs and Mount, 1978) pointed out that the microcosm idea was basically sound, but that its implementation was still problematic because of difficulties in replicating the natural system.

Because of the increased complexity of microcosm-test procedures, it would be difficult to use them successfully on a routine basis in a large-scale assessment program. However, they may be useful in small-scale assessments, such as in studies of selected stream reaches. In such cases, they would probably be used at sites where special toxicant problems may occur, as indicated by initial single-species tests and by chemical analyses.

Battery Approach

The precision and accuracy of any scientific study generally are improved both by replication of a single type of experiment and by sequential attack on the question using a variety of experiments. Given the uncertainties surrounding the assessment of biological responses to constituents in the aquatic environment, replication and sequential testing merit special consideration for toxicity testing. Interpretation of toxicity-test results involves comparisons of toxicant concentrations that elicit biological responses and actual toxicant concentrations in the natural environment. If a battery approach is used, there is a presumed improvement in the reliability of this comparison because it is based on a more diverse data base than it would be if only one test were used. The data base will be especially diversified if the selected test organisms represent different trophic levels (Maciorowski and Clarke, 1980).

Several researchers have reported good results in applications of the battery approach. Dutka and Kwan (1982) found that four bacterial screening procedures they tested were each characterized by particular sensitivity patterns and could not be readily correlated with each other. If only one procedure were used, it could give misleading

information. Further evidence that independent toxicity tests may give misleading results when interpreted alone was provided by data from Lake Ontario (Dutka and others, 1986). Plotkin and Ram (1984) tested the effects of sanitary landfill leachate on fish, daphnids, algae, and bacteria. The responses were very different among the different organisms, and not reliably predictable on the basis of measured concentrations of toxicants in the leachate. They concluded that toxicity assessments should be based on multiple tests with organisms from different trophic levels. Three test species—bacteria, oyster embryos, and amphipods—tested by Williams and others (1986) showed considerable variation in sensitivity to toxic sediments. The authors emphasized that a diversity of toxicity-testing procedures was important for evaluating sediment toxicity.

Diversification of toxicity tests by using the battery approach may improve the reliability of statistical treatment by providing a broader data base. Multiple trophic levels may be tested, providing a more complete characterization of the community. The different strengths of a diverse array of tests may be used in complementary fashion.

Although the benefits of diversification are recognized, there are also disadvantages. The sensitivities of different test species vary considerably but, as shown in figure 2, they may all be insufficient to permit positive detections of contaminant concentrations commonly found in natural waters. Hence, consistent negative results from all tests of a battery do not necessarily lead to a firm conclusion that there is no toxicant problem. If, on the other hand, some of the tests in the battery produce positive results while others do not, the composite result may be ambiguous and interpretation may be especially difficult.

The previously discussed arguments against microcosm procedures may also apply to the battery approach. A multispecies approach, whether microcosm or battery, is more complex and more costly than a single acute-lethality test. Is it simply a more costly means to arrive at the same answer? The cost problem is an especially important consideration for designing a toxicity-test approach for large-scale assessments.

A variation of the battery approach is a sequential screening procedure (Slooff, 1985) (fig. 3). A rapid test, such as a bacterial luminescence test, is used as an initial screening tool. If ambient substances produce stress responses in the initial screening test, a second test at a higher level of biological organization is performed. This process may proceed through several levels of biological organization to assess toxicity effects. Cairns, who had earlier joined with others in advocating the sequential test approach (Cairns and others, 1978), later argued that sequential testing, if done at all, should not proceed from lower to higher levels of biological organization (Cairns, 1983). He pointed out a "lack of substantive evidence that one can accurately predict the response at higher levels of biological organization from the single-species tests."

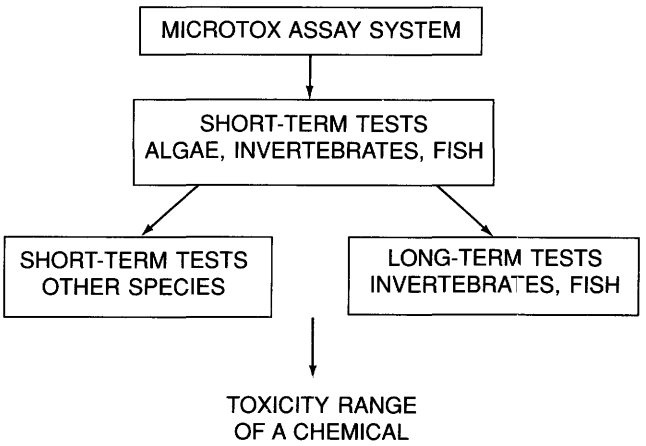

Figure 3. General scheme for sequential toxicity screening.

Sediment Toxicity Tests

Conventional toxicity tests involve assessment of the effects of toxicants dissolved or suspended in water. However, large numbers of aquatic organisms, including many of the test species listed in table 3, reside in or on bottom sediments. Others are exposed directly to sedimentary materials because they are benthic or deposit feeders. Furthermore, sediments are an important repository for many contaminants that may be released to overlying water. These factors support the argument that toxicity assessments of aquatic systems should include exposure of test organisms to contaminants contained in the sediments. This is the rationale leading to relatively recent development and application of sediment toxicity tests, mostly in the marine environment (Tsai and others, 1979; Swartz and others, 1980; Chapman and Fink, 1984; Tietjen and Lee, 1984; Long and Chapman, 1985; Swartz and others, 1985; Mearns and others, 1986). Freshwater studies include those of Prater and Anderson (1977), Laskowski-Hoke and Prater (1981), Cairns and others (1984), Malueg and others (1984a, b), Nebeker and others (1984), and Schuytema and others (1984).

Although sediment toxicity tests involve investigation of contaminants associated with the sediments, the exposure route is not necessarily through direct contact between organism and sediments. Any one of three different exposure routes are possible in the experimental design (Chapman, 1987):

1. Exposure to whole, intact sediments. This is generally the preferred exposure route, especially if the test species inhabits the sediments. The test enclosure contains contaminated sediments and water. The test species may be either benthic or pelagic (that is, it may inhabit either sediments or water). Exposure may be through direct contact with sediments, ingestion of sedimentary materials, or contact with overlying water that carries desorbed or resuspended contaminants.

2. Exposure to a sediment elutriate (suspended or liquid phase). The test enclosure contains water that has previously contacted the contaminated sediments (as in a sediment-water slurry) for a specified time, then is filtered or centrifuged. Because of desorption or resuspension, water contains contaminants previously associated with sediments. The sediments themselves are not added to the test enclosure. This may be the method of choice if it is not practical to add sediments to the test system, or if the toxicological response involves aqueous contact (for example, respiration; Chapman and Brinkhurst, 1984).
3. Exposure to a sediment extract. The test enclosure contains water to which sediment extract is added. The extract is an organic carrier solvent containing some of the contaminant that has transferred to it during an extraction procedure similar to procedures used for chemical analyses. Thus, the water-extract mixture contains contaminants previously associated with sediments. The sediments themselves are not added to the test enclosure. Again, this method may be appropriate if it is not practical to add sediments to the test system. It is applicable only for readily extractable nonionic organic contaminants (Chapman, 1987).

Comparisons of the elutriate and whole-sediment exposure routes by Chapman and Fink (1984) showed some discrepancies in results. Toxicity responses in some cases were caused only by elutriate exposure, and in other cases only by whole-sediment exposure. Ideally, whole-sediment exposure should be used in combination with either sediment-elutriate or sediment-extract exposure to obtain more complete toxicity information.

Sediment toxicity-test procedures can be used for acute or chronic testing with any of the common test species, whether benthic or pelagic (Nebeker, Cairns, Gakstatter, and others, 1984). Swartz and others (1985) monitored mortality and sublethal responses of amphipods exposed directly to sediments in static test beakers. Control survival was 95 percent, and the organisms were quite sensitive to contaminants amended to the sediments. An example of the elutriate exposure method is the three-chamber-recirculation apparatus used by LeBlanc and Suprenant (1985) to test the effects of contaminated sediments on fathead minnows, daphnids, and midges. Responses of test organisms were closely correlated with the degree of chemical contamination of sediments.

Some verification studies of sediment tests have produced favorable results. Field validation by Swartz and others (1980) showed good correlations between sediment toxicity, as determined by amphipod responses, and 18 biological and geochemical variables on a pollution gradient on the Palos Verdes Shelf (California). Mearns and others (1986) conducted an interlaboratory comparison of an amphipod sediment toxicity test and obtained results that led them to recommend wider use of the test. Control survival was greater than 90 percent in five laboratories, and the laboratories were in close agreement on toxicity ranking and mean responses. Acute toxicity of sediment extracts as determined by bacterial bioluminescence (Schiewe and others, 1985) correlated with total concentrations of aromatic hydrocarbons, chlorinated hydrocarbons, and naphthalenes in the sediments.

The disadvantages of sediment toxicity testing should also be considered prior to incorporation in a long-term study plan. First, the introduction of sediments (or their elutriates or extracts) into the test system complicates the chemistry of the system and increases the likelihood of secondary variable effects. Second, although a more complex system may be more realistic, it also makes interpretation of test results more difficult. Third, the sensitivity of tests involving exposure to whole, intact sediments may be inferior to that of conventional tests because of the likelihood that the contaminant must move through the aqueous or suspended phase before affecting the test organism. Most quality-assurance work with sediment techniques indicates good sensitivity, but it has also been noted that sediments tend to ameliorate toxicity of contaminants in the system (Chapman and others, 1982b; Graney and others, 1984). Fourth, relatively little work with sediment toxicity tests has been done, especially for freshwater systems. Therefore, the documentation of methods and availability of comparative data are limited. Finally, work with sediment increases the complexity of collecting samples for testing and of performing the tests.

The importance of sediments, both as a habitat for biological species and as a reservoir for many xenobiotic substances, suggests that sediment tests should not be overlooked in designing a toxicity-testing study. The most productive approach for most studies, provided funding and personnel are adequate, is to implement a suitable combination of sediment and water tests supplemented with chemical analysis of the same sediment and water media.

Biochemical Tests

The toxicity of heavy metals and organic compounds to aquatic biota is very commonly attributable to direct or indirect effects of the toxicant on enzyme activity, biochemical functions, or membrane integrity (Neff, 1985). Therefore, it is reasonable to expect that one of the most sensitive indices of contaminant stress would be a change in enzyme activity, enzyme synthesis, or biochemical composition. Toxicity-test methods that use this approach have been developing rapidly in recent years. Most of the research has been done with fish.

It is logical to look at the effects of toxins on biochemical processes as a first step in toxicity testing. Biological responses to stress may be thought of as a series, propagating through increasingly complex levels of organization (Jenkins and Sanders, 1986). Biochemical changes

are very early in the series; for practical purposes, they are initial responses. Furthermore, they are common to many different kinds of organisms. The biochemical changes may elicit subsequent responses at the cellular, organ, organism, population, and, finally, community levels. But as one proceeds along this scale of propagation, the variability of response increases because of increasing secondary effects due to individual tolerances and environmental factors. Thus, both the sensitivity and the reproducibility of biochemical-response measurements are likely to be greater than those of other types of toxicity testing.

Biochemical changes tend to be rapid and very responsive to causative factors, in part because they are initial responses to stress. For example, a study by Kurelec and others (1977) showed that mixed-function oxygenase activities in Blennideae fish in the Adriatic Sea increased by nearly an order of magnitude within a few days after an oil spill. This kind of quick and dramatic response is not uncommon.

There are many possible variations of the biochemical assay approach to toxicity testing. Generally, they involve exposure of the living organism or tissues of the organism to the contaminant and measurement of relatively short term changes in enzyme activity, biochemical composition of blood and tissues, or production of detoxification proteins such as metallothioneins or mixed-function oxygenase systems. Biochemical techniques of monitoring responses may be applied in either a laboratory setting (test organisms in an enclosure) or a community survey (capture and analysis of native organisms).

Metallothioneins are proteins that have a high binding capacity for divalent metal cations. They have been identified in many species of fish and other animals, and they are thought to play an important role in detoxification of several metals, including silver, gold, cadmium, mercury, copper, and zinc (Neff, 1985). Exposure to elevated concentrations of such elements should stimulate production of metallothioneins (Roch and others, 1982; Thompson and others, 1982; Sanders and others, 1983; Sanders and Jenkins, 1984).

Mixed-function oxygenase (MFO) systems might be considered the counterpart of metallothioneins with respect to detoxification of organic contaminants. MFO systems include a group of enzymes that initiate metabolization of numerous lipophilic organic compounds, rendering them more water soluble and therefore more available for excretion. They have also been identified in many fish species (Neff, 1985). Various studies have demonstrated increased MFO activity as a result of exposure to organics in the environment (Payne, 1976; Stegeman, 1978; Lech and others, 1982; Foureman and others, 1983). Ironically, the fish does not necessarily benefit from this increased MFO activity. Instead, there may actually be an increase in toxicity, owing to the production of intermediates that are carcinogenic (Hinton and others, 1981; Tan and others, 1981).

Enzymes may be affected directly or indirectly by toxicants, usually resulting in an increase in enzyme activity. Increased activity in glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase, two indicators of liver pathology, may be induced by elevated concentrations of carbon tetrachloride (Casillas and others, 1983) or sewage discharges (Weisner and Hinterleitner, 1980). Some enzymes are affected specifically by certain pollutants. One of these is delta amino levulinic acid dehydratase (ALAD), which is contained in blood erythrocytes and is important in the formation of hemoglobin, cytochrome, and peroxidase. Its activity may be sharply inhibited by lead in the blood (Hodson and others, 1978). Concentrations as low as 10 µg/L (micrograms per liter) can produce significant inhibition of erythrocyte ALAD in rainbow trout (Hodson and others, 1977). In other species, such as carp and white suckers, the ALAD activity was a less sensitive indicator of lead contamination.

In addition to enzymes, the production and activity of various biochemical substances in the blood and tissues may be affected by exposure to toxicants. In fish blood, some of the substances most frequently affected by pollutants are cortisol, glucose, proteins, lactic acid, pyruvic acid, and cholesterol. In tissues, some of the most frequently affected substances are glycogen, proteins, lipids, collagen, glutathione, and ascorbic acid.

Biochemical responses to stress are common to all types of organisms and thus can be used as bioindicators of toxicity in a wide range of environmental situations. Responses vary according to the type and concentration of the causative agent (Jenkins and Sanders, 1986). These stress-response relations currently are not well understood, but as more information about them becomes available, the usefulness of biochemical testing to identify particular types of contaminant problems should improve.

Biochemical analysis offers considerable promise for application in large-scale studies. The number of possible methods is almost limitless, considering the number of toxic agents in the environment and the number of biochemical responses caused by those toxicants. In general, biochemical testing has the same advantages as bacterial tests and offers the additional advantage of greater sensitivity.

The primary disadvantages of the biochemical approach are (1) limited knowledge of the correlation between biochemical responses and deleterious effects on fish populations and (2) biochemical variability caused by a great number of environmental variables other than toxicant concentrations (Neff, 1985). The latter problem is significant for biochemical techniques because there are so many factors that can have biochemical effects, and the resulting biochemical fluctuations can be dramatic. The stress of capture and handling can be an especially important controlling factor. As methodology development proceeds, some of these difficulties may be overcome and biochemical techniques will become increasingly useful.

Alternatives to Laboratory Toxicity Testing

Toxicity tests conducted in static, flow-through, or microcosm systems represent just some of various types of biological study that could fill the need for biological data to assess water quality. These types of tests most directly address the question of effects of contaminants. However, certain alternative approaches may be implemented to provide somewhat different kinds of information about toxicity.

One alternative is to omit test enclosures altogether and conduct ecological surveys in the study basin. The sites for such surveys may be selected to represent a range of contaminant conditions in the basin. Chemical data may be used to make appropriate site selections. The survey data can provide information about community structure (species present and relative abundance of each), diversity (numbers of species relative to numbers of individuals), and biomass (total abundance of biota, regardless of taxa). This information provides a useful complement to chemical data collected at the same sites and times (Lafont, 1984; Long and Chapman, 1985).

Ecological surveys avoid some of the problems of prolonged incubation of enclosure organisms in artificial enclosures. They are not dependent on representation of an entire system by a limited number of species, and they are not subject to inaccuracy due to unnatural conditions in a controlled environment. More analytical work (for species identification and organism counts) is required, but standard methods are generally available. The high costs of this analytical work may be offset by savings in labor and equipment costs.

The principal disadvantage of ecological survey analysis is that the lack of control on environmental variables limits the ability of the researcher to infer cause-effect relations from the results. Even if trends are found (either temporal or spatial), they are not sufficient in themselves to show cause and effect. However, they do provide clues that may be used to identify areas that merit further toxicity testing and other biological investigation. Conversely, if no trend is found, the study makes a significant statement that would be lacking if only the chemical data were available.

A second alternative is to install artificial substrates in the natural environment. As in the ecological surveys, site locations are selected to represent a range of contaminant conditions. The colonization of the artificial substrates by natural biota is observed and measured after being left undisturbed in the system for a specified time period. Colonization rates and species composition of the established community may reflect contaminant effects. Several possible variations of this general procedure are possible, including the incubation of natural substrates from the stream system or the measurement of loss of organisms from a substrate (such as loss due to drift of attached benthos). Like the ecological survey approach, this procedure has the advantages and disadvantages of being conducted in the natural environment. However, it is somewhat less natural and more controlled than the ecological survey because of the use of standardized colonization surfaces and a limited and rather arbitrary colonization time.

A third alternative is to introduce test organisms in the natural environment in enclosures, such as cages, that prevent their escape but allow exposure to all elements of the environment just as if they were free living. After a specified incubation period, mortality and (or) sublethal changes may be determined and compared with control organisms incubated in unaffected sites. This procedure is similar to a standard toxicity test because it uses test organisms selected primarily for their sensitivity and practicality for study and entails enclosure of the organisms for a limited incubation time. On the other hand, it is similar to ecological surveys or artificial substrate measurements because the organisms are exposed to natural conditions.

Toxicity-test procedures that use relatively large test organisms such as mollusks may be supplemented by tissue analysis to determine bioaccumulation rates. If contaminants are accumulated over time in the biological tissue, the organisms function as integrators of time-variable contaminant inputs and thus allow detection of substances that might be missed by analysis of constituents in water. However, bioaccumulation rates are highly dependent on properties of the constituents and characteristics, particularly lipid content, of the biological species. They are also susceptible to changes in environmental conditions such as pH, temperature, sediment characteristics, and organic carbon concentrations.

Overview of Test-Type Differences

The differences among test types are reflected in their particular strengths and limitations. As a summary of the preceding discussion, table 20 lists major test types and some of the reasons why each type might be selected or deselected for water-quality-assessment purposes.

FIELD AND INTERLABORATORY VERIFICATION OF TOXICITY TESTS

Field verification of laboratory tests is one means of obtaining quality-assurance information about toxicity-test procedures. In some cases, additional quality assurance has been achieved through interlaboratory comparisons.

A 1983 symposium (Boyle, 1985) was dedicated to validation of laboratory toxicity-testing methods, with emphasis on verification of microcosms and mathematical models by comparison with field data. Most of the authors reported favorably on their verification results (Adams and others, 1985; Giddings and Franco, 1985; Harrass and Taub, 1985; Levy and others, 1985; Portier, 1985).

Other verification results reported at the 1983 symposium revealed some inconsistencies between laboratory

Table 20. Arguments for and against use of selected toxicity-test procedures

Arguments for:	Arguments against:
1. Acute tests, single species (static or flow-through).	
o Good documentation of methods.	o Unnatural; single-species responses in limited enclosure do not reflect species interactions in natural community.
o Extensive data base of results from diverse systems.	o Variable sensitivity; species sensitive to some toxicants, insensitive to others.
o Endpoint (mortality) readily detected and monitored.	o Relatively long culture times required; may cause mortality or other problems.
o Results reported in standardized, unambiguous LC50 format.	
o Good control and replicability of test conditions.	
2. Chronic tests, single species.	
o Relatively good documentation of methods.	o Responses often subtle; may be difficult to detect or monitor.
o Large data base of results from diverse systems.	o Format for reporting results not well standardized; can be ambiguous.
o Good control and replicability of test conditions.	o Unnatural; single-species responses in limited enclosure do not reflect species interactions in natural community.
o Sensitivity of sublethal responses greater than that of mortality (sometimes by orders of magnitude).	o Variable sensitivity; species sensitive to some toxicants, insensitive to others.
	o Long culture times required; may cause mortality or other problems.
3. Laboratory microcosms, or multispecies tests.	
o Relatively good documentation of methods.	o Complicated setup increases cost per test and decreases number of replicates possible.
o Ample data base of results from other studies.	o Multispecies situation increases complexity and likelihood of secondary variable effects and problems of laboratory culture.
o Good control and replicability of test conditions.	o Responses often subtle; may be difficult to detect or monitor.
o Replication of natural community; responses incorporate species interactions.	
4. Bacterial tests.	
o Simplicity and rapidity; many replicates possible.	o Very poor sensitivity to most toxic agents.
o May be conducted in local laboratories; eliminates need for shipping samples and delay between sampling and testing.	o Poor reproducibility; sensitive to slight changes in test conditions or characteristics of bacterial populations.
o Avoids many problems of lab enclosures and long culture times.	
o Good representation of general toxicity because luminescence response is dependent on common biochemical pathways.	
5. Sediment tests.	
o Sediments are natural repository of many toxicants.	o Variable pathways of exposure through sediments; need to use different types of tests in combination.
o Many aquatic organisms exposed to higher toxicant concentrations through sediments than through water.	o Sediments in test system complicate chemistry; makes interpretation more difficult.
o Sediment-water system more representative of natural conditions.	o Sediments may ameliorate toxicity or decrease bioavailability of toxicants.

Table 20. Arguments for and against use of selected toxicity-test procedures—Continued

Arguments for:	Arguments against:
	o Relatively small data base, especially for freshwater systems.
	o More difficult sample collection, treatment and laboratory set-up.
6. Biochemical or physiological tests.	
o High sensitivity to most toxicants; biochemical or physiological changes one of first responses to environmental stresses.	o Relatively new field; limited (but rapidly increasing) data base and documentation of methods.
o Simplicity; usually only involves collection of blood samples that may be stored or shipped with little risk of deterioration.	o Especially sensitive to secondary variable effects.
o Avoids problems of laboratory culture.	
o Many replicate analyses possible.	
o Responses readily detectable and may be reported in unambiguous manner.	
7. Community surveys in impacted and unimpacted areas.	
o Study of natural system; avoids problems of artificial culture.	o No control over environmental variables and associated secondary effects.
o Use of naturally occurring species.	o Poor reproducibility over time and space because of changing conditions and variable sample-collection techniques.
o Simplicity; no laboratory setup required; low cost for supplies and equipment.	o Time-consuming fieldwork, species identification, and individual counting required; high labor cost.
o Reflection of effects of long-term exposure rather than limited and arbitrary exposure time.	
8. Artificial substrates in natural environment.	
o Study conducted in natural system; representation of natural conditions.	o No control over environmental true variables and associated secondary effects.
o Use of naturally occurring species.	o Difficult to compare data over time and space because of changing conditions and different species.
o Simplicity; no laboratory setup required; low cost for supplies and equipment.	o Time-consuming fieldwork, species identification, and individual counting required; high labor cost.
	o Species limited to those that can colonize artificial substrate.
	o Risk of loss or destruction of test substrates.
9. Incubation of test organisms in cages in natural system.	
o Study conducted in natural system; true representation of natural conditions.	o No control over environmental variables and associated secondary effects.
o Test species may be selected among most suitable bioindicator species.	o Difficult to compare data over time and space because of changing conditions.
o No laboratory setup required; relatively low cost for supplies and equipment.	o Species generally limited to those that have relatively large size and limited mobility.
	o Limited number of species tested; usually only one.
	o Risk of loss or destruction of test enclosures.

and field data. Experiments by deNoyelles and Kettle (1985) to determine the effects of atrazine on phytoplankton indicated that short-term laboratory bioassays are reasonably effective in representing natural stress responses for about 24 hours, but that later the responses become unnatural owing to conditions not representative of the natural environment. The effects of fluorine on various trophic levels were monitored in both laboratory static toxicity tests (Finger and others, 1985) and experimental ponds (Boyle and others, 1985). Algae and invertebrates were sensitive to fluorine in the laboratory enclosures, but in the ponds, two fish species were more sensitive than either the algae or the invertebrates.

Varying degrees of support for laboratory-testing methods have been expressed by other researchers who have compared laboratory and field data. Greene and others (1976) reported that replication of a natural lake community by the algal assay test was excellent, and they derived an equation for predicting chlorophyll-*a* concentrations in the lake on the basis of assay results. Kallqvist (1984) also reported good results with algal assays, and suggested that the patterns of growth of experimental and control cultures can be used to classify natural waters into one of five categories of toxicant and nutritive conditions. Larsen and others (1986) compared three procedures—single-species tests, microcosm, and experimental pond—to examine the effects of atrazine on eight species of algae. Good replication was reported; 50-percent inhibition of photosynthesis, respiration, and algal biomass occurred in all three systems at atrazine concentrations within the range of 100 to 150 µg/L.

A study by Mount and others (1984) included diverse procedures whose results could be used to evaluate the validity of ambient toxicity testing. The effects of discharges from a municipal sewage treatment plant, a refinery, and a chemical company on the Ottawa River, Ohio, were investigated. In addition to ambient toxicity tests of waters from various sites downstream from the discharge points, the study included effluent toxicity tests, dye studies to describe dilution characteristics of the effluents, and in situ toxicity tests with fish in cages. It also included surveys of periphyton, phytoplankton, zooplankton, benthos, and fish. Positive toxicity-test results were obtained with some of the ambient samples collected from sites likely to be affected by the effluents. The authors reported that these results correlated reasonably well with aquatic community measurements, as determined by regression analyses. However, they also acknowledged that various complexities, such as year-to-year variations and toxicities upstream from effluent inputs, made interpretation difficult. They also pointed out the need to collect extensive and diverse biological data. At least two test species from different trophic levels should be used, and biological surveys should include monitoring of many segments of the aquatic community.

A similar study by the same group (Mount and Norberg-King, 1985) of an Ohio stream included additional comparison of ambient toxicity-test results with community measurements. However, no verification of the test results was possible because no toxicity was shown by the tests. The stream receives discharge from a chemical resins plant, but even the undiluted effluent was not appreciably toxic to the test organisms.

Recent studies of sediments from embayments of Puget Sound, Wash. (Long and Chapman, 1985; Chapman, 1986), included three phases: (1) sediment chemical analysis to determine concentrations of three metals, polychlorinated biphenyls, and polynuclear aromatic hydrocarbons, (2) sediment toxicity tests, using bulk sediments and an amphipod test species, and (3) surveys of the infaunal communities at the sample sites. This "sediment quality triad" approach showed good correlation among results from all three phases of the study. However, the correspondence was not nearly as consistent on a station-by-station basis. The authors recognized weaknesses in the data sets, and complexities such as contaminant interactions, that contributed to inconsistencies. The general implication was that if there is good correspondence among toxicity, chemistry, and community measurements, conclusions about the toxicity problem can be drawn, but that lack of correspondence does not lead to converse conclusions. The deviation from "expected" results may be attributable to single or combined effects of innumerable environmental factors that are not accounted for in the triad analysis.

Various authors (Leeuwangh, 1978; Lee and Jones, 1983; Sadler, 1983; Kimball and Levin, 1985; Livingston and Meeter, 1985; Thorp and Gloss, 1986) have pointed out that the reliability of laboratory tests is significantly influenced by differences between laboratory and natural systems in their physical, chemical, and ecological conditions. This might be termed a "secondary variable" effect, a biological response caused by unnatural conditions in the laboratory environment that is not related to additions of the tested contaminant. One of the most important secondary variables is pH. Numerous studies have demonstrated effects of pH changes on toxicity of contaminants to test organism (Nasu and Kugimoto, 1981; Suloway and others, 1981; Giddings and others, 1983; Lee and Jones, 1983; Eloranta and Halttunen-Keyrilainen, 1984; Graney and others, 1984; Michnowicz and Weaks, 1984). In some cases, detrimental effects on the test organism caused by secondary variables may exceed toxic effects of the contaminant (Leeuwangh, 1978). Secondary variables may affect laboratory results in either direction. For example, low metal methylation rates may lead to underestimates of toxicity in laboratory tests (Benson and Summons, 1981), but the inability of mobile organisms to escape in a test enclosure may lead to overestimates of toxicity (Kimball and Levin, 1985).

Table 21 summarizes reports of field verification of laboratory testing procedures. No clear consensus appears about whether laboratory tests are good indicators of contaminant effects in a natural system. However, there does seem to be good agreement between microcosm tests and natural systems monitoring.

Interlaboratory or "round robin" tests have been conducted on several occasions to evaluate the reliability and precision of particular toxicity-test procedures. Favorable results of such a test were reported by Davis and Hoos (1975), who compared data from seven laboratories on determination of pentachlorophenate (PCP) toxicity to salmonid fishes. The range of 96-hour LC50 values reported for rainbow trout was 48 to 100 µg/L, calculated by the log-probit estimate, and 47 to 106 µg/L, calculated by nomographic analysis. The results were considered by the authors to show good consistency or, where disparities occurred, to be explainable by variations in the physical or chemical conditions of the test. An interlaboratory comparison of determinations of silver and endosulfan toxicity to a polychaete worm was conducted by Pesch and Hoffman (1983). Mean 28-day LC50 values reported were 165±52 µg/L for silver and 106±24 µg/L for endosulfan. These results were considered to demonstrate low variability among laboratories. A sediment toxicity test was evaluated by Mearns and others (1986), based on participation by five laboratories. The laboratories all reported better than 90-percent survival in control sediments. There was also at least 80-percent agreement among the laboratories on the rank order of toxicity for three endpoints (survival, emergence, and reburial), and on the mean survival in the test sediments. Because of the narrow range of toxicity of the tested samples, the interlaboratory results did not show close agreement as to whether the sediments should be classified as toxic or nontoxic.

Somewhat less optimistic results of interlaboratory comparisons were reported by Nebeker (1982). Six laboratories participated in a round-robin experiment, based on *Daphnia magna* renewal life-cycle test results for silver and endosulfan toxicity. Four of the laboratories reported 48-hour EC50 values for silver within a range of 0.6 to 2.9 µg/L, but the other two laboratories reported much higher values (8.4 to 55 µg/L). The explanation given by the author for this discrepancy was that the two laboratories reporting the higher EC50 results used harder water in their experiments. The range of reported EC50 values for endosulfan was 158 to 720 µg/L. A number of difficulties interfered with consistent results in this test, and the author concluded that "the *Daphnia magna* renewal life-cycle test was not validated as a routine, easily conducted test method."

A protocol for interlaboratory testing of a microcosm toxicity-test procedure was described by Taub (1985). Precaution in standardizing variables that might cause inconsistencies among different laboratories was emphasized. Preliminary analysis was done by comparing replicate control groups in a single laboratory. As stated by the author, "the ability to obtain repeatable results within a single laboratory is a necessary prerequisite to testing for reproducible results in different laboratories." These data could be used not only to evaluate the precision of replicate experiments but also to determine if the biological activity in the microcosms is sufficient to assess toxicant stresses. The analysis indicated both good replication and adequate biological activity.

In general, interlaboratory comparisons have indicated that toxicity tests can produce better precision than might be expected, given the extreme natural variability that is characteristic of nearly all biological systems. However, reports of good precision do not necessarily imply high accuracy. For example, if a toxicant is present at concentrations below the sensitivity of the test organisms, the concurrence of all participating laboratories in a negative result for this toxicant would simply emphasize the detection limitations of the test. Interlaboratory comparison data cannot be used to evaluate sensitivity, representativeness, or relevance of the test method (criteria 1, 2, and 6 of table 2).

Table 21. Comparison of different types of toxicity tests

[Symbols indicate whether study reported that there is good agreement ("+"), poor agreement ("o"), or variable agreement (both symbols) between the two types of tests]

Compared types				
Static and Flow-through	Static and Microcosm	Static and Natural	Microcosm and Natural	Reference
		+		Adams and others, 1983
			+	Adams, Werner, and others, 1985
o				Cherry and others, 1980
o				Biesinger and others, 1982
	+ o			Thorp and Gloss, 1986
		o		Kettle and others, 1980
		+		Marshall, 1978
	+			Hansen and Garton, 1982
		o		Kimball and Levin, 1985
	+			Davies and Woodling, 1980
			+	Portier, 1985
	+	+	+	Giddings and Franco, 1985
		+		Greene and others, 1976
			+	Harrass and Taub, 1985
		o		Kay and others, 1984
	+	+	+	Larsen and others, 1986
			+	Levy and others, 1985
		+		Norberg-King and Mount, 1986
		+		Mount and others, 1984
		o		Maciorowski and Clarke, 1980
		+ o		Sadler, 1983
			+	Blanck, 1985

Test types are defined as follows:

Static: Static, acute, single-species test in laboratory

Flow-through: Continuous-flow, acute, single-species test in laboratory

Microcosm: Enclosed, simulated community, in either lab or field

Natural: Natural aquatic system. Data obtained by field monitoring, such as ecosystem surveys, measurements of community metabolism, etc.

SUMMARY

Contaminants in the aquatic environment are of concern for biological reasons. They are potentially harmful to native aquatic organisms and to nonaquatic organisms, including humans, that use the water resource in some way. Organisms that are not directly affected by the contaminants may suffer indirectly through food-web transfers. Therefore, biological analyses add breadth and relevance to a water-quality monitoring program. As one of several types of biological analysis, toxicity testing produces information about direct impacts of contaminants on aquatic biota. Combined with chemical analyses, toxicity-testing procedures may provide an opportunity to correlate biological variables with contaminant concentrations and chemistry. However, there are important limitations to application of toxicity-testing procedures to a large-scale assessment program. These include sensitivity limitations, difficulties in representing the natural environment, response variability among test organisms, and secondary variable effects.

To further summarize the information in this report, I refer back to the five questions raised in the "Introduction." The questions are repeated below, along with my brief answers to them, based on the foregoing review.

1. What are the characteristics and applications of different types of toxicity tests?

A wide variety of test types are documented by published reports. Procedures can be distinguished primarily on the basis of four criteria: (1) test species, (2) endpoint, (3) test enclosure, and (4) test substance or toxicant. Ambient tests can be done with water, sediments, or sediment extracts. The test organisms used can be limited to a single known species, or may include mixed species. Applications vary according to test type and study objectives. Applications such as the use of test results as a preliminary survey for planning further toxicological research or as a verification of other types of water-quality monitoring involve relatively little risk of error. More risky applications are those that require extrapolation of test results to more complex systems.

2. What are the advantages and disadvantages of different types of test procedures, particularly with reference to application in large-scale water-quality assessments?

Acute tests, including bacterial tests, are straightforward and rapid, but they often have poor sensitivity and are not representative of natural situations. Chronic tests commonly offer better sensitivity and are more realistic, but endpoints are usually more subtle and more difficult to monitor. Chronic responses are highly susceptible to changes in environmental conditions. Multispecies tests, such as large microcosm experiments, might extend the general applicability and sensitivity range beyond those of a single-species test, but their use in large-scale studies is limited by their design complexities.

Compared with laboratory toxicity tests, field-oriented procedures more closely represent natural situations and are not as subject to error due to artificial experimental conditions or species selection. There are other important sources of error, however, including natural biological variability, sampling inconsistencies, and lack of control over environmental conditions. Where possible, it is advantageous to employ both laboratory and field procedures, in addition to chemical analyses, to provide a diverse data base for thorough quality assessment.

3. Do the results of toxicity tests accurately reflect environmental conditions and the probable effects of contaminants on biota in natural systems?

It is rare that a microcosm situation, as used in most toxicity tests, can truly mimic the natural system it is intended to represent. Although many documented toxicity tests may be considered reliable measures of toxic effects under specified conditions, they cannot replicate the complexity or variability of the natural system. A positive result suggests the potential for a toxicant problem in the natural system but does very little to predict the nature of the probable response. A negative result does not prove the absence of a problem or potential problem in the natural system.

Notwithstanding the difficulty of representing natural situations and stress responses, toxicity tests can be useful to indicate the occurrence of a condition that may be of concern, and to suggest possible directions of further research. They may be especially applicable when used in conjunction with other water-quality and hydrologic monitoring data. Hence, failure to closely mimic natural stress responses is not necessarily critical. The need to accurately represent a natural system depends on the intended use of the test results.

4. Will different toxicity tests result in different conclusions about existing toxicant problems in the environment?

Yes. Because of widely varying sensitivities among different species and among different test conditions, two tests of different types are unlikely to give similar results. This is the principal argument for implementation of a battery of tests in a large-scale assessment program. Procedures included in the battery may be selected so that the test species represent different trophic levels and the tests complement each other with respect to sensitivity to different toxic agents. More extensive and diverse data would be produced, and false negative results would be less likely (assuming that a positive response on just one test in the battery would constitute an overall positive result). However, there would be substantially greater labor and monetary expenditure than would be required for a single test.

5. Is there a particular type of test, with respect to selection of test species, test substance (ambient or artificial), and test medium (water or sediment), that can be applied in

standardized format, with consistently reliable results, over a broad range of aquatic systems and environmental conditions?

No. The variety of toxicant situations and the diversity of biological communities and environmental conditions among different study sites are so great that no single existing test method is universally applicable and reliable.

Many criteria are important for selection of an appropriate toxicity test. Sensitivity and capability to represent species responses in the natural environment are the most critical. Other criteria that are especially important for selecting tests to use in large-scale studies are reproducibility, simplicity of procedures, availability of background information, documented methodology, and cost. Each type of test has its advantages, but none meets all of the selection criteria adequately to be considered useful as a universal test.

The best prospects for future development of a universally applicable test are in the area of biochemical assays. There are many sensitive biochemical methods, most of which are simple and applicable with native organisms. Most important of their advantages is that the biochemical changes monitored are initial responses to environmental stimuli, and the responses are common to nearly all organisms.

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APPENDIX: TOXICITY-TEST PROCEDURES

As indicated in this report, many biological species and different procedures are used in toxicity testing. Detailed descriptions of the methods for each type of test are not given here, largely because they would only duplicate descriptions that are readily available in the literature. Publications that contain method descriptions for each of various test species and test types are listed in appendix tables 1 and 2.

As an aid to readers who are unfamiliar with the toxicity-testing approach in general, some concepts and common features of test procedures are discussed below.

Most toxicity-test procedures require enclosure of organisms, either in the laboratory or in the natural environment. Each enclosure functions as a simple microcosm to demonstrate an environmental effect. Functional, anatomical, and (or) behavioral characteristics of test organisms are monitored simultaneously in each system. Changes of biological variables in experimental systems are compared with those in control systems. The experimental systems contain known concentrations of the test substance, varying from a concentration that is not expected to be bioeffective to a concentration that is equal to or greater than any concentration likely to be found in nature. Such a test gradient is intended to bracket the minimum bioeffective concentration. The control systems contain no introduced toxicants, but all other conditions are identical (or as close as possible) to those of the experimental systems.

Ideally, any stress response by the test organisms will be attributable to the introduced toxicant rather than to other conditions of the experiment. Therefore, it is important to monitor such properties as temperature, dissolved oxygen, pH, water hardness, and light in the experimental and control media. To the extent possible, these conditions should be controlled to maximize (1) their consistency among all enclosures, (2) their stability throughout the experiment, and (3) their representation of natural conditions.

Some species are amenable for use in virtually any type of toxicity test, and their responses may be monitored by means of any of a variety of acute or chronic endpoints. For other species, the choices of test type and endpoints are more limited. Bacteria and phytoplankton, for example, cannot be used in acute lethality studies because of the impracticality of monitoring mortality in these microscopic organisms. Some population metabolic rates, such as bioluminescence or primary productivity, can be readily measured by standard methods, and are used as endpoints for species of microbiota.

Most applications of toxicity tests call for replication of experimental and control systems such that there are at least duplicate systems for each toxicant concentration tested, and for the controls. When macroscopic animals are used as test organisms, there are recommended limits to the number and biomass of organisms in each test chamber. These limits vary according to species and type of test, but for small invertebrates and early life stages of fish, they are on the order of 1–5 g/L (grams per liter) (live weight) and 10 organisms per chamber.

The duration of the test may vary from a few minutes for some bacterial tests to several months for some chronic life-cycle tests with invertebrates and fish. Acute tests with invertebrates and fish commonly have specified durations of 48 or 96 hours. The length of the test, and the requirements of the species, are important in determining whether or not to feed the test organisms during the test. The question of feeding presents somewhat of a dilemma. Lack of feeding may be stressful to the organisms and increase their susceptibility to toxicant effects (Nebeker and others, 1983). However, feeding introduces another potential variable that could affect test results. Some of the references in appendix table 1, in particular those marked with an "S," contain considerable discussion of feeding techniques and effects.

There are various possible field microcosm or mesocosm configurations, including limnocorrals (large enclosures set in a lake or other water body) and experimental ponds. The general approach for these microcosm studies is similar to that for laboratory studies in that the biological effects of an introduced toxicant in experimental systems are measured against comparable biological activity in control systems. Field microcosm units usually are very large and contain a multispecies community that closely replicates the natural community they represent. Because they are set in the natural environment, their physical and chemical conditions are not under the control of the experimenter, but they are likely to mimic conditions in the larger system.

Other field toxicity studies involve biological monitoring of organisms exposed to existing conditions in the natural environment, rather than to a test substance introduced by the researcher. The test organisms may be naturally occurring biota in their natural habitats, biota that colonize some artificial habitat emplaced in the natural system, or introduced species held in any type of enclosure that allows environmental exposure while preventing escape of the organisms. Monitoring usually is done at selected sites that represent a known or suspected gradient of toxicant concentrations. For example, sampling sites might be located upstream and at various distances downstream from a point source. Chemical analyses of water and sediments from the same sites and times provide complementary data that are useful for interpretation of the biomonitoring results.

Appendix Table 1. Partial list of publications containing detailed descriptions of methods for species-specific toxicity tests.

[Within a taxonomic group, "X" indicates species used. Both acute and chronic test methods are applicable to invertebrate and fish species, and are indicated by "A" and "C," respectively. "S" indicates description of special tools or auxiliary procedures that can facilitate or modify the method. Special feeding techniques or requirements are included in the special procedures]

BACTERIA

Reference	*Photobacterium phosphoreum*	Others
Bitton, 1982	X	X
Bulich, 1979	X	
Burton & Lanza, 1985		X
Coleman & Qureshi, 1985	X	X
De Zwart & Slooff, 1983	X	
Dutka & Kwan, 1981	X	X
Dutka & Kwan, 1982	X	X
Dutka & others, 1983	X	X
Freeman, 1986	X	X
McFeters & others, 1983	X	X
Schiewe & others, 1985	X	
Seyfried & Morgan, 1985		X
Slabbert & Grabow, 1986		X
Vasseur & others, 1984	X	

PROTOZOANS

Reference	*Chilomonas paramecium*	*Tetrahymena pyriformis*
Honig & others, 1980	C	
Slabbert & Morgan, 1982		C

GREEN ALGAE

Reference	*Selenastrum capricornutum*	*Scenedesmus quadricauda*	Others
Aly & others, 1984		X	
Bartlett & others, 1974	X		
Christensen & others, 1979	X		
De Vries & Hotting, 1985			X
Freeman, 1986	X		
Gaur & Kumar, 1986	X		
Giddings & others, 1983	X		
Joubert, 1983	X		
Kuivasniemi & others, 1985	X		X
Miller & others, 1978	X		
Ordog, 1982	X	X	X
Payne & Hall, 1979	X		X
Trotter & Hendricks, 1976			X
Van Coillie & others, 1983	X	X	X

Appendix Table 1. Partial list of publications containing detailed descriptions of methods for species-specific toxicity tests—Continued

[Within a taxonomic group, "X" indicates species used. Both acute and chronic test methods are applicable to invertebrate and fish species, and are indicated by "A" and "C," respectively. "S" indicates description of special tools or auxiliary procedures that can facilitate or modify the method. Special feeding techniques or requirements are included in the special procedures]

Reference	Lemna minor	Eichhornia crassipes	Others
MACROPHYTES			
Bishop & Perry, 1981	X		
Hartman & Martin, 1985	X		X
Kay & others, 1984		X	
King & Coley, 1985	X		
Lockhart & others, 1983	X		
Wang, 1986	X		

Reference	Limnodrilus hoffmeisteri	Tubifex tubifex	Others
OLIGOCHAETES			
American Public Health Association & others, 1985	AC	AC	
Bailey & Liu, 1980			A
Chapman & Mitchell, 1986			A
Chapman & Brinkhurst, 1984	A	A	A
Chapman & others, 1982a	A	A	
Chapman & others, 1982b	A	A	

Reference	Daphnia magna	Ceriodaphnia spp.	Other Daphnia
CLADOCERANS			
Adams and Heidolph, 1985	C		
American Public Health Association & others, 1985	AC		
Barera & Adams, 1983	A		
Bowman & others, 1981	A		
Buikema & others, 1980	ACS		
Cowgill & others, 1985	S		
Geiger & others, 1980			AC
Gersich & Mayes, 1986	A		
Goulden & others, 1982	ACS		
Horning & Weber, 1985		C	
Jop & others, 1986			S
Keating, 1985			S
LeBlanc & others, 1983	C		
McNaught & Mount, 1985		C	
Mount & Norberg, 1984		C	
Nebeker, 1982	AC		
Norberg & Mount, 1985a		S	
Peltier & Weber, 1985	A		A
Taylor, 1985		S	

Appendix Table 1. Partial list of publications containing detailed descriptions of methods for species-specific toxicity tests—Continued

[Within a taxonomic group, "X" indicates species used. Both acute and chronic test methods are applicable to invertebrate and fish species, and are indicated by "A" and "C," respectively. "S" indicates description of special tools or auxiliary procedures that can facilitate or modify the method. Special feeding techniques or requirements are included in the special procedures]

	OTHER CRUSTACEANS		
Reference	Amphipods	Decapods	Others
American Public Health Association & others, 1985	AC		
Abel, 1980	A		
Abel & Garner, 1986	A		
Arthur, 1980	AC		
Bowman & others, 1981	A		A
Buikema & others, 1980			AC
Martin & Holdich, 1986	A		A
Prater & Anderson, 1977			A
Swartz & others, 1985	AC		
Thorp and Gloss, 1986		A	
Graney & Geisy, 1987	A		

	INSECTS		
Reference	Chironomidae spp.	Hexagenia spp.	Others
Anderson, 1980	A		
Batac-Catalan & White, 1983	C		
Bowman & others, 1981			A
Darville & Wilhm, 1984	C		
Fremling & Mauck, 1980		ACS	
Prater & Anderson, 1977		A	
Nebeker, Cairns, & Wise, 1984	A		

	MOLLUSKS	
Reference	Corbicula spp.	Other bivalves
Harrison & others, 1984	A	
Dauble & others, 1985	C	
Paparo & Sparks, 1977		C
Rodgers & others, 1980	A	

	OTHER INVERTEBRATES
Reference	NEMATODES
Tietjen & Lee, 1984	X
Samoiloff & others, 1980	X
Haight & others, 1982	X

Appendix Table 1. Partial list of publications containing detailed descriptions of methods for species-specific toxicity tests—Continued

[Within a taxonomic group, "X" indicates species used. Both acute and chronic test methods are applicable to invertebrate and fish species, and are indicated by "A" and "C," respectively. "S" indicates description of special tools or auxiliary procedures that can facilitate or modify the method. Special feeding techniques or requirements are included in the special procedures]

	FISH			
Reference	*Pimephales promelas*	*Salmo gairdneri*	*Lepomis macrochirus*	Others
American Public Health Association & others, 1985	AC			AC
Alexander & others, 1978	A			
Cleveland & others, 1986	AC	AC	AC	AC
Feder & Collins, 1982	C			
Gersich & Mayes, 1986	A			
Horning & Weber, 1985	C			
Mason, 1981	C		C	C
McKim & others, 1987		C		
Nebeker & others, 1985		AC		
Norberg & Mount, 1985b	C			
Peltier & Weber, 1985	A			A
Phipps & Holcombe, 1985	A	A	A	A
Sprague, 1973	A	A	A	
van der Schalie, 1980			C	
Westlake & van der Schalie, 1977			C	
Murty, 1986	ACS	ACS	ACS	ACS

Appendix Table 2. Partial list of publications containing detailed descriptions of methods for different types of toxicity tests

SMALL LABORATORY ENCLOSURES (Acute or Chronic)		
Reference	Static	Flow-through
Alexander & others, 1978	X	X
American Public Health Association & others, 1985	X	X
Bishop & Perry, 1981		X
Bowman & others, 1981	X	
Brungs, 1973		X
Buikema & others, 1980	X	X
Geiger & others, 1980	X	
Gersich & Mayes, 1986	X	
Hansen & Tagatz, 1980		X
Horning & Weber, 1985	X	
Mason, 1981	X	X
Meador & others, 1984		X
Mount & Brungs, 1967		X
Mount & Norberg, 1984	X	
Nebeker, 1982	X	
Nebeker & others, 1984	X	
Norberg & Mount, 1985b	X	
Peltier & Weber, 1985	X	X
Phipps & Holcombe, 1985		X
Sprague, 1973	X	X
Thurston & others, 1985		X
Birge & others, 1979		X
Iwan & Cella, 1981		X
Gruber & others, 1980		X
Meador & others, 1984		X
Wuerthele & others, 1973		X

LABORATORY MICROCOSMS		
Reference	Aquaria	Artificial streams
Adams, V.D., & others, 1985	X	
Black & others, 1973	X	
Giddings, 1986	X	
Giddings & Franco, 1985	X	
Graney & others, 1984		X
Hansen & Tagatz, 1980	X	
Harrass & Taub, 1985	X	
Hedtke, 1984	X	
Honig & Buikema, 1980		X
Levy & others, 1985	X	
Portier, 1985	X	
Rodgers & others, 1980		X
Shriner & Gregory, 1984		X
Stay & others, 1985	X	
Taub & Crow, 1978	X	
Taub & others, 1983	X	
Westlake & van der Schalie, 1977	X	
Yasuno & others, 1985		X

Appendix Table 2. Partial list of publications containing detailed descriptions of methods for different types of toxicity tests—Continued

FIELD MICROCOSMS

Reference	Experimental ponds and streams	Limno-corrals	Others
deNoyelles & Kettle, 1985	X		
Giddings & Franco, 1985	X		
Hedtke & Arthur, 1985	X		
Herman & others, 1986		X	
Kaushik & others, 1985		X	
Kaushik & others, 1986		X	
Robinson-Wilson & others, 1983	X		
Marshall & Mellinger, 1980			X
Wilde & Parrott, 1984			X

FIELD INCUBATION PROCEDURES

Reference	Artificial subtrates	Caged organisms
Beak & others, 1973	X	
Foe & Knight, 1987		X
Rice & White, 1987		X
Perkins, 1983	X	
Leland & Carter, 1985	X	

OTHER TEST TYPES

Reference	Sediment tests	Biochemical and physiological tests	Electronic and computerized monitoring
LeBlanc & Suprenant, 1985	X		
Long & Chapman, 1985	X		
Malueg & others, 1984a	X		
Prater & Anderson, 1977	X		
Schiewe & others, 1985	X		
Bitton, 1982		X	
Graney & Geisy, 1987		X	
Hinton & others, 1973		X	
Katz, 1979		X	
Neff, 1985		X	
Wong & others, 1982		X	
Besch & others, 1977			X
Fisher & others, 1982			X
Gruber & Cairns, 1981			X
Gruber & others, 1980			X
Kleerekoper, 1977			X
Maki, 1979			X
Morgan, 1977			X
Poels, 1977			X
van der Schalie, 1980			X

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