Sediment Toxicity Assessment

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LEWIS PUBLISHERS
Boca Raton Ann Arbor London Tokyo

Library of Congress Cataloging-in-Publication Data

Burton, G. Allen
Sediment toxicity assessment/edited by G. Allen Burton
p. cm.
Includes bibliographical references and index.
ISBN 0-87371-450-4
1. Sediments (Geology)--Evaluation.. 2. Pollution--Environmental aspects--Evaluation. 3. Toxicity testing. 4.Toxicology. I. Burton, G. Allen.
QE471.2.S39 1992
628.1'61--dc20 91-44273
CIP

Direct all inquiries to CRC Press, Inc. 2000 Corporate Blvd., N. W., Boca Raton, Florida 33431

LEWIS PUBLISHERS, INC.
121 South Main Street, Chelsea, MI 48118

PRINTED IN THE UNITED STATES OF AMERICA
1 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

CHAPTER 10

Freshwater Benthic Toxicity Tests

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INTRODUCTION

Benthic macroinvertebrates, as a group, are often the optimal assessment tool in determinations of sediment toxicity. As discussed in Chapters 4 and 5, macroinvertebrate community structure indices have been used for many years as effective and sensitive indicators of ecosystem pollution.[1-3] A substantial data base exists on macroinvertebrate responses to xenobiotics, nutrients, and other physicochemical perturbations. In addition, life cycles and habitat and culturing requirements are known for a number of species that play a major role in the function of many aquatic ecosystems, such as *Chironomus* (midges), *Tubifex* (aquatic earthworms), *Hyalella* (scuds), *Gammarus* (scuds), and *Hexagenia* (mayfly nymphs).[4-6] Their intimate contact with bottom sediments and interstitial and overlying waters for extended periods of their life cycle increases the likelihood for adverse effects occurring in the presence of contaminated sediments. Benthic macroinvertebrates fill a multitude of ecological niches: functioning as prey, predators, herbivores, omnivores, collectors, gatherers, shredders, filter feeders, and thus interacting with multiple trophic levels, controlling energy/nutrient/organic matter cycling dynamics in many ecosystems.[7-9]

HISTORY

Sediment toxicity testing began with freshwater benthic macroinvertebrates, namely the mayfly, *Hexagenia limbata*,[10] and the midge, *Chironomus tentans*,[11,12] in

1977. These studies using acute (96 hr) and chronic exposures (11 to 28 days) indicated survival, growth, and emergence were related to bulk sediment contaminant concentrations.[10-15] Most sediment testing in the late 1970s and early 1980s[10,13,16-19] was focused on concerns of dredging of contaminated sediments and the potential impact of dredge material (acute effects) on water quality and biota.[14,16-21] The U.S. Army Corps of Engineers (COE) and the U.S. Environmental Protection Agency (EPA) developed guidance for the testing of dredge and fill materials in marine systems, using three appropriate species and conducting acute exposures in whole sediments, suspended sediments, and elutriate (water-extractable) fractions.[20] Exposure periods and test phases used in dredging evaluations were designed to mimic dredging conditions: that is, short-term perturbations primarily involving suspended solids and water-extractable toxicants. Some have questioned the realism of the recommended test conditions and the sensitivity of many of the test species.[22]

During the past 10 years the research and literature concerned with assessing sediment contamination has expanded substantially.[23] Laboratory studies involving benthic invertebrate species have varied widely in their experimental design, species selection, endpoints of toxicity, and manipulation of sediments. These approaches, and their associated strengths and weaknesses, will be discussed in the following sections. Standardization of methods is advantageous for some study objectives and regulatory usage and has recently begun within the American Society for Testing and Materials (ASTM), Subcommittee E47.03 on Sediment Toxicology.[24] Standard guides for whole-sediment toxicity testing with the midges, *Chironomus tentans*, *C. riparius*, and the amphipod, *Hyalella azteca*, were approved in 1990. Draft methods also exist for *Daphnia* and *Ceriodaphnia* sp., *Hexagenia*, and oligochaete acute- and short-term chronic toxicity testing.

ASTM Subcommittee E47.03 on Sediment Toxicology was established in May of 1987 and is one of 11 subcommittees in the ASTM Committee E47 on Biological Effects and Environmental Fate. The goal of the sediment subcommittee is to develop guides for assessing the bioavailability of contaminants associated with sediments. These guides are used to evaluate the toxicological hazard of contaminated sediment, soil, sludge, drilling fluids, and similar materials. The subcommittee initially decided to develop guides, not test methods, because most testing procedures for sediment have been recently developed. Eventually, test methods can be developed from the guides when definitive procedures for a particular test are established. Over the past four years the subcommittee has gained ASTM approval for three sediment toxicity testing guides.

TEST CONDITIONS

As discussed in Chapters 1 and 3, the sediment environment is very complex, consisting of a quasi-stable physical system in which numerous physicochemical and microbiological gradients exist and interact. Inorganic and organic substances, both

of natural (e.g., carbonates, oxyhydroxides, humics, low-molecular-weight fatty acids) and anthropogenic (e.g., arsenic, cadmium, copper, lead, polycyclic aromatic hydrocarbons, biphenyls, dibenzodioxins, pesticides) origin, partition between sediments, interstitial water, overlying water, and resident biota. Many sampling and laboratory manipulations can have a dramatic impact on partitioning (Chapter 3) and, thereby, affect toxicity responses in the test species. Some obvious conditions that may affect results include overlying water quality, sediment/water contact time, exposure period, and exposure phase.

Overlying Water Quality

Sediment/water contact time in sediment toxicity assays may exert substantial effects on overlying water quality and therefore organism response.[25] Sediment oxygen demand (biochemical and chemical) can be significant in some sediments rich in nutrients and reduced substances,[26] requiring aeration of the overlying water.[27] The dissolution of sediment components, such as carbonates, may elevate hardness,[28] which would affect the availability of some metals such as Cd, Cu, and Pb. Disturbance of redox gradients and increased oxygenation may result in reduced levels of acid volatile sulfides (AVS), and thus the possible release of available metal.[29] Exposure conditions have consisted of static,[28] recirculating,[10,17,30] static-renewal,[31] and flow-through systems,[17,28] and system comparisons have shown significant differences in toxicity response in some studies.[28,32] Static and flow-through tests of sediments from Waukegan Harbor were equally toxic to amphipods, but when other sites were included in the statistical analyses, flow-through exposures provided greater survival. This was likely a result of the flushing of contaminants from overlying water that had desorbed from the sediment.[28] Ingersoll and Nelson[28] recommend whole-sediment tests be conducted with low turnover rates (<4 chamber volume per day) to maintain more consistent overlying water quality and to reduce the flushing of contaminants from the exposure system.

In whole-sediment assays, a 1:4 ratio of sediment to water has been common;[31,32] however, the Prater-Anderson recirculating system has been used at a ratio of 1:9.5.[10,18] The 1:4 ratio probably originated from the COE elutriate preparation procedure. The interaction between the sediment and the overlying water in the test chambers, and the ratio of sediment to overlying water in the test chambers, may influence the availability of the contaminants. Tests may need to be conducted with the range of environmental conditions expected in the overlying water of sediment. For example, water hardness or pH of the overlying water may alter sediment toxicity.[22] Stemmer et al.[33] investigated the influence of sediment volume and surface area on the toxicity of selenium-spiked sediment to *Daphnia magna*. Varying surface area within a constant 1:4 ratio of sediment to water did not alter daphnid survival; however, a decrease in the sediment-to-water ratio (1:8) and an increased surface area decreased survival of the test organisms. These results indicate that test conditions that deviate substantially from more conventional test methods using a 1:4

sediment-to-water ratio may affect contaminant availability, and standardizing sediment-to-water ratios may be necessary in order to make comparisons between species.

Test Phase

Perhaps the most important issue in sediment toxicity testing is the appropriate sediment phase to test. Sediment phase can be categorized as follows: extractable (solute other than water) phase, elutriate (water-extractable) phase, interstitial water phase, whole sediment, and in situ assays. Each has associated strengths and weaknesses that prevent the recommendation of any one phase to meet all study objectives. The issues discussed previously regarding sediment integrity and contaminant sorption and desorption are particularly pertinent when attempting to interpret assay responses between different sediment phases. These considerations are summarized in Table 1.

Few studies have compared test phases as treatments.[34,35] Some studies have compared phases, but using different assays,[34,36] which does not allow a true comparison of phase effects on toxicity. The elutriate phase has been shown to be more toxic[37] and less toxic[34,35,38] than other phases. In studies of four areas in the Great Lakes[34,38] and one stream in Ohio,[35] the elutriate fraction was always less toxic than whole-sediment assays using the same endpoints. Some sediment toxicity effects are only associated with the whole phase.[39] Interstitial waters, however, were more toxic or of equal toxicity to whole sediment.[35] The greater toxicity may be due to elevated ammonia concentrations[40] that are diluted in overlying waters in whole-sediment assays. This, however, may be an artifact of pH shifts, which may increase when interstitial waters are isolated, thereby increasing ammonia toxicity. Higher metal concentrations have been observed in interstitial waters compared to elutriates.[19,41] The sediment interstitial water toxicity test was developed for evaluating the potential in situ effects of contaminated sediment on aquatic organisms. Once the interstitial water or elutriate samples are isolated from the whole sediment, the toxicity testing procedures are similar to effluent toxicity testing with nonbenthic species, described in Chapter 8. If benthic species are used as test organisms, they may be stressed by the absence of sediment.[42] Methods for sampling interstitial water have not been standardized. Isolating sediment interstitial water has been accomplished using several methods, including centrifugation, squeezing, suction, and dialysis.[43,44]

Knezovich et al.[45] stated that organism morphology, ecological niche, feeding mechanism, and physiology will determine toxicant, uptake, pathway, and, thus, hazard. For example, oligochaetes are sediment ingesters, while many benthic and epibenthic species are filter feeders,[46] and thus are exposed to interstitial and overlying waters to varying degrees.[46,47] It is likely that no consistent relationship between relative toxicity of all interstitial, elutriate, and whole-sediment assays will ever exist, due to the multitude of physicochemical and biological process variables.

Some acute toxicity assays using benthic invertebrates have been conducted in sediment-free systems such as interstitial water, elutriate phase, or spiked waters.[34-40,48] Most benthic test organisms (such as *Hyalella azteca, Chironomus* sp., or *Hexagenia limbata*) require substrate contact or burrowing capabilities during their life cycles;[4,5] the absence of sediment may induce artificial and perhaps stressful conditions.[42,49] Stress has been observed in exposures greater than 48 h by decreased control survival or cannibalism. The relationship of this unnatural stress factor on acute-effect-level determinations is unknown, but should be considered.

EXPOSURE CONDITIONS

Given the sensitive and tenuous nature of sediment integrity, exposure conditions are particularly crucial in determining contaminant behavior and organism or community response. Parameters of concern include time of exposure, feeding, and both the chemical and physical environment (e.g., light, temperature, dissolved oxygen).

Time

Most marine and freshwater sediment toxicity testing has been limited to acute testing where exposure periods typically were 15 min for Microtox®, 48 hr for cladocerans, 96 hr for fish, and 4 to 10 days for amphipods, oligochaetes, chironomids, and Ephemeroptera. Greater sensitivity to toxicants occurs with extended exposure.[17,28,50-53] Concentrations of contaminants in sediments may not be acutely lethal, but may interfere with the ability of an organism to develop, grow, or reproduce. However, only a limited amount of freshwater subchronic and chronic toxicity testing has been conducted and has usually consisted of amphipod reproduction and growth (28 day), oligochaeta growth (10 day), and chironomid growth and emergence (10 to 15 day). Debate continues in aquatic toxicology over the definitions, adequacy, or relationships between acute, subchronic, and chronic toxicity testing.[54-56] Early life stage tests that monitor fecundity and growth are often more sensitive than survival studies using adults.[57] However, long-term survival in chronic toxicity tests may be more sensitive than other endpoints.[58] The sensitivity of molecular and cellular endpoints is greater than community structure and ecosystem function endpoints; however, determining their ecosystem relevance is much less clear (Figure 1). While there are obvious advantages in conducting subchronic tests (e.g., shorter testing period, thus less resource intensive, allowing more testing), chronic tests do not require extrapolation from shorter test exposure periods.[55,56] In addition, different lethal or sublethal biochemical endpoints can be studied, so both types of assays are useful and necessary.

Table 1. Sediment Toxicity Exposure Phases

Phase	Strengths	Weaknesses	Routine Uses
Extractable Phase (XP) (solutes vary)	Use with all sediment types Sequentially extract different degrees of bioavailable fractions Greater variety of assay endpoints available Determine dose-response	Ecosystem realism: bioavailability unknown, chemical alternation	Rapid screen Unique endpoints, so component of test battery
Elutriate Phase (EP) (water extractable)	Use with all sediment types Readily available fraction Mimics oxic toxic environmental process Large variety of assay endpoints available Methods more standard Determine dose-response	Ecosystem realism: only one oxidizing condition used; only one solid: water ratio; exposure for extended period of one phase condition which never occurs in situ or never occurs in equilibrium in situ Extract conditions vary with investigator Filtration affects response, sometimes used	Rapid screen Endpoints not possible with WS Dredging evaluations
Interstitial water (IW)	Direct route of uptake for some species Indirect exposure phase for some species Large variety of assay endpoints available Methods of exposure more standard Determine dose-response Sediment criteria may be determined	Can't collect IW from some sediments Limited volumes can be collected efficiently Optimal sampling method unknown, constituents altered by all methods Exposure phase altered chemically and physically when isolated from WS Flux between overlying water and sediment unknown Relationship to and between some organisms, uncertain: burrowers, epibenthic, water column species, filter feeders, selective filtering, life cycle vs. pore water exposure	Rapid screen Endpoints not possible with WS Initial surveys Sediment criteria

Whole sediment (WS)	Use with all sediment Relative realism high Determine dose-response Holistic (whole) vs. reductionist toxicity approach (water, IW, EP, and XP) Sediment quality criteria may be determined Use site or reconstituted water to isolate WS toxicity	Some physical/chemical/microbiological alteration from field collection Dose-response methods tentative Testing more difficult with some species and some sediments Few standard methods Indigenous biota may be present in sample	Rapid screen Chronic studies Initial surveys Sediment criteria
In situ[a] (IS)	Real measure integrating all key components, eliminating extraneous influences Criteria may be determined Resuspension/suspended solids effects assessed	Few methods and endpoints Not as rapid as some assay systems Mesocosms variable Predation by indigenous biota	Resuspension effects Intensive system monitoring Sediment criteria

[a] Organisms exposed in situ in natural systems, pond/stream mesocosms, or lake limnocorrals.

Source: Burton[152]

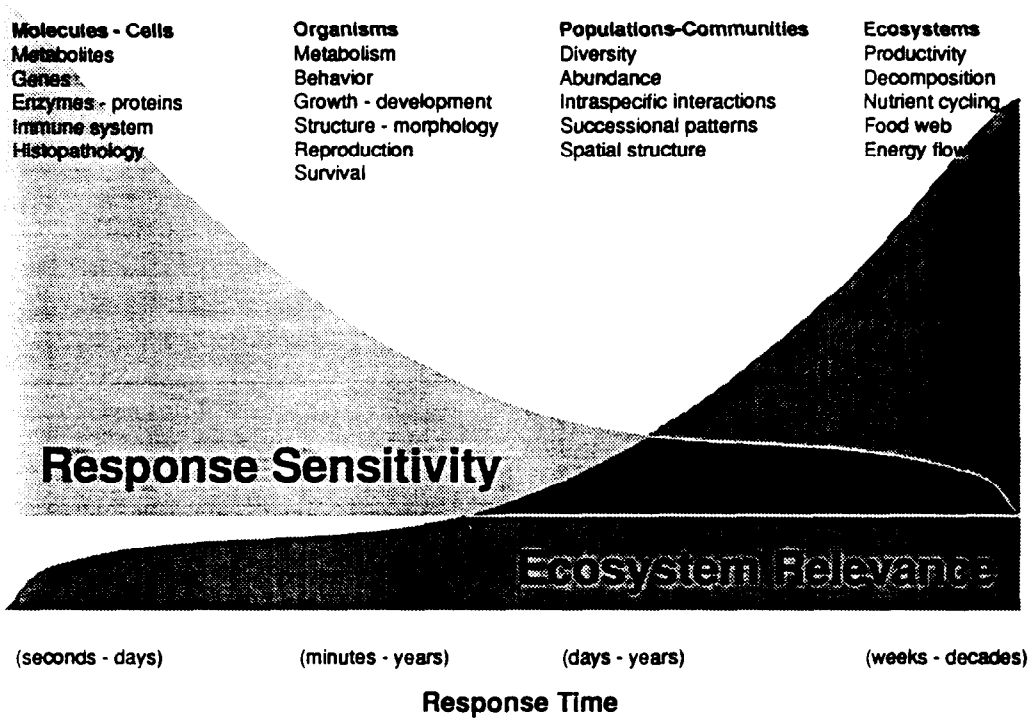

Figure 1. Levels of biological organization. (From Burton, G. A., Jr. "Assessing Toxicity of Freshwater Sediments," *Environ. Toxicol. Chem.* 10:1585–1627 (1991). With permission.)

Feeding

The same testing factors (feeding, physical and chemical conditions) that have been recognized as important in controlling toxicity responses in effluent pure-chemical and water column assays,[59] are also important in assays of sediment toxicity. Feeding may alter toxicant exposure and elimination rates and must be considered (see Chapters 12 and 13). However, some different considerations and parameters do exist, such as water quality changing through time.

In longer exposures, supplemental food is often added to the test chambers. Without the addition of food, the test organisms may starve during longer exposures, but the addition of the food may alter the availability of the contaminants in the sediment.[60] Furthermore, if too much food is added to the test chamber or if mortality of test organisms is high, mold-bacterial growth will develop on the sediment surface. If test organisms are fed in whole-sediment tests, the amount of food should be kept to a minimum. If food accumulates on the sediment or if a mold or bacterial growth is observed on the surface of the sediment, feeding should be suspended for 1 or more days. Detailed records of feeding rates and the appearance of the sediment should be made daily.

Species Selection

The species tested should be selected based on (1) their behavior in sediment (e.g., habitat, feeding habits), (2) their sensitivity to test material(s), (3) their ecological relevance, (4) their geographical distribution, (5) their taxonomic relation to indigenous animals, (6) their acceptability for use in toxicity assessment (e.g., a replicable and standardized method, ease of test method), (7) their availability, and (8) their tolerance to natural geochemical sediment characteristics such as grain size. Many species that might be appropriate for sediment testing do not meet these criteria because, historically, an emphasis has been placed on developing testing procedures for water column exposures. Test species should not be collected at or near a disposal site, since these populations may have developed an enhanced resistance to contaminant perturbations. Unfortunately, culturing methods and testing procedures have not been developed for many benthic animals.[61]

Sensitivity is related to the degree of contact between the sediment and the organism. Feeding habits, including the type of food and feeding rate, will control the dose of contaminant from sediment.[46] Infaunal deposit-feeding organisms can receive a dose of sediment contaminants from three sources: interstitial water, whole sediment, and overlying water. Benthic invertebrates may selectively consume particles with higher organic carbon concentration and higher contaminant concentrations. Organisms in direct contact with sediment may also accumulate contaminants by direct adsorption to the body wall or exoskeleton or by absorption through the integument.[45] Thus, estimates of bioavailability will be more complex for epibenthic animals that inhabit both the sediment and the overlying water. Tests with elutriate samples measure the water-soluble constituents potentially released from sediment to the water column during dredge disposal operations.

Geochemical Characteristics

Natural geochemical properties, such as sediment texture, may influence the response of infaunal animals in sediment tests. It is important to select test organisms that have a wide range in tolerance to natural sediment properties. The natural geochemical properties of test sediment collected from the field need to be within the tolerance limits of the test species. The limits for the test species should be determined experimentally. Controls for such factors as particle size and organic carbon should be run if the limits of the test animal are exceeded in the sediments. The effects of sediment characteristics such as grain size and organic carbon concentration can either be addressed experimentally using toxicity tests or be addressed using normalizing equations (see Chapter 9). Studies of the influence of additional "noncontaminant" factors, such as sediment moisture, organic content, and water quality (e.g., hardness, pH, Eh, ammonia), on the response of test animals

are required to differentiate between effects resulting from the influence of natural sediment characteristics and effects caused by contaminants.

The route of exposure may be uncertain, and data generated in sediment toxicity tests may be difficult to interpret, if normalizing factors for bioavailability are unknown. Bulk sediment chemical concentrations need to be normalized to factors other than dry weight. For example, concentrations of nonpolar organic compounds might be normalized to sediment organic carbon content, and metals normalized to acid volatile sulfides.

Indigenous Animals

Indigenous animals may be present in field-collected sediments.[28] An abundance of the same species, or of species taxonomically similar to the test species in the sediment sample, may make interpretation of treatment effects difficult. Previous investigators have inhibited the biological activity of sediment with heat, mercuric chloride, antibiotics, or gamma irradiation. Gamma irradiation is probably the most desirable method because it causes the least alteration in either the physical or chemical characteristics of the sediment.[44] Further research is needed to determine the effects on the bioavailability of contaminants from treating sediment to destroy indigenous organisms before testing.

TESTS OF SEDIMENT TOXICITY

The benthic community is comprised of several taxonomic levels of organisms, and many have been used in toxicity assessments, including bacteria, protozoans, nematodes, bryozoans, oligochaetes, amphipods, gastropods, pelecypods, insects, and periphyton.[23] The following section will focus those assays that have been reported several times to be useful in sediment toxicity assessments.

Bacteria

Microbial assays can be divided into testing groups of either indigenous communities or laboratory cultured strains and assay endpoints that are biochemical (such as enzyme activity, bioluminescence, lipopolysaccharides, muramic acid, and ATP content) or other metabolic processes (such as growth, uptake, respiration, substrate transformation, viability, and microcalorimetry). These endpoints have been measured by a multitude of methods, primarily in studies of water, wastewater, and soil systems, and have been applied to sediment systems, to some degree.

Biomonitoring by EPA has not routinely included testing of the microbial community. The Toxic Substances Control Act recommends premanufacturing testing of chemical effects on several microbial processes. Microbial testing is also a component of new-product testing under the Federal Insecticide, Fungicide, and

Rodenticide Act. Microtox® has been listed by the EPA as a supplemental test to use in Tier 1 screening tests in the *Technical Support Document for the Water Quality-Based Toxics Control Approach.*[62] However, limited use is actually made of microbial toxicity tests in any of the EPA program activities.[63,64]

Microbial responses have been recommended as early warning indicators of ecosystem stress[65] and as a means of establishing toxicant criteria for terrestrial and aquatic ecosystems.[64] The resulting changes at the species level should be accompanied by changes in respiration and/or decomposition rates.[65] The usefulness of monitoring the microbial community is due, in part, to its ability to respond so quickly to environmental conditions (e.g., toxicant exposure) and the major role they play in ecosystem biogeochemical cycling processes and the food web.[66,67]

When investigating chronic toxicity and other early warning indicators of toxicant stress, stimulatory effects are often noted at low toxicant concentrations in fish, cladoceran, algal, macrophyte, and microbial indicator assay responses.[38,68,69] This phenomenon, known as hormesis, is common when using microbial and photosynthetic organisms as indicators. Stimulatory effects can be attributed to nutrients, adapted microbial communities, the Arndt-Schultz phenomenon, or feedback mechanism disruption.[70,71] Elevated structure and function responses were initial stress indicators, probably reflecting a disruption of normal feedback mechanisms controlling microbial nutrient dynamics and species interactions.[71] Stimulation or inhibition of activity may also result when carbon or nutrient substrates are altered, so that one enzyme system, e.g., alkaline phosphatase activity, is stimulated while another, such as galactosidase activity, is inhibited.[72] When comparing test samples with reference samples, inhibitory *and* stimulatory effects should be regarded as possible perturbations.

Pure microbial culture systems used in assessments of sediment extracts include Microtox® *(Photobacterium phosphoreum)*[73] and *Spirillum volutans*,[74] *Escherichia coli*,[75] *Nitrobacter* sp.,[76] *Azotobacter vinelandii*,[77] *Aeromonas hydrophila*,[78] *Pseudomonas fluorescens*,[79] and *P. putida*.[80] In most comparative surveys *Spirullum* was the least sensitive to toxicity, but not in other studies.[81] Pure-culture studies with bacteria and fungi have demonstrated that sensitivity to metals is equal to or less than plant or animal systems.[82]

Microtox® testing has recently been incorporated into sediment toxicity test strategies (Chapter 15) and was originally used in marine sediment assessments.[73] Some toxicity has been attributed to the extraction of natural organics.[83] The insensitivity of Microtox® to some elements and compounds may result from an inappropriate diluent (ionic strength adjustor).[40,73,84,85] Interstitial water in large-grain contaminated sediments was more toxic than fine-grain sediments; however, the opposite was observed for solvent-extracted sediments.[85] Numerous comparisons of Microtox® sensitivity to pure compounds and effluents with *Daphnia* sp. and fish (primarily *P. promelas*) indicate similar effect levels,[86,87] and Microtox® was generally more sensitive than other microbial tests.[88] In three of the four sediment comparison studies,[34,89,90] Microtox® was very sensitive and discriminatory of

sediments contaminated with a wide variety of synthetic organic and metal compounds. In a fourth study where the primary toxicant was ammonia, interstitial water effects were observed on *P. promelas*, *C. dubia*, and *S. capricornutum*, but not Microtox®.[40] Recently, a whole-sediment exposure method using *Photobacterium phosphoreum* was presented and appeared to be more sensitive to hydrophobic chemicals than the elutriate Microtox® assay.[91]

Metabolic processes such as methanogenesis, sulfate reduction, denitrification, and carbon dioxide evolution, or enzymes involved in key metabolic systems such as dehydrogenases, alkaline phosphatase, and glucosidase, have been measured in contaminated sediments.[68,72,92-99] Some of these processes were depressed, whereas other processes were stimulated.[66] Discrimination of contaminated and noncontaminated sediments requires several response endpoints.[68,97,98] Burton and Stemmer[68] evaluated five stream profiles across the U.S. in which several indigenous oxidoreductase and hydrolase enzyme activities in waters and sediments were compared to in situ chemical concentrations, biological community structure, and laboratory test animals. At all five sites significant relationships were observed between indigenous enzyme activities and in situ conditions, indicating toxicant effects, natural spatial variation, and food web interactions. Activity of β-galactosidase indicated significant relationships in 80% of the studies to 37.5% of the biological and chemical stream parameters measured. β-glucosidase and dehydrogenase activity were significant indicators of stream conditions in 60% of the studies, whereas *C. dubia* reproduction (water only) was related in 50% of the studies to 22.5% of the stream parameters. Hydrolases were also effectively used to define sediment spatial variance in creosote-contaminated sediments.[33]

In summary, studies to date have demonstrated that indigenous microbial enzyme activity and bioluminescence (e.g., Microtox®) are generally as sensitive as fish and invertebrate toxicity assays to metals, some organics, and contaminated sediments. Microbial assays are effective at discriminating degrees of sediment contamination[34,89] and are related to in situ conditions at most sites. Assays of microbial processes and natural assemblages of microorganisms tend to be superior to pure-culture test systems. Bacteria should be considered in perturbation studies, since microbes play such an important role in energy flow and ecosystem functioning. However, since bacteria reproduce and adapt relatively quickly, pollutant effects must be greater than macrofaunal responses to be ecologically relevant.

Protozoa

Protozoan tests of sediment contamination have primarily evaluated overlying waters or the elutriate phase. Pratt and Cairns[100] grouped freshwater protozoa into six functional groups based on food requirements: dissolved mineral nutrients; bacteria and detritus; algae, bacteria, and detritus; diatoms; dissolved organics;

and, rotifers and protozoans. Protozoans feeding on the "bacteria and detritus" component comprise the majority of genera. Protozoan communities are a complex structure of herbivores, carnivores, omnivores, and detritus feeders.[101] The majority of species are cosmopolitan and tolerate a wide range of freshwater quality.[4] Protozoa play an important role in food web dynamics and the "microbial loop".[1,4] Holophytic and saprozoic protozoans are producers that use dissolved nutrients and are food for meiofauna, as are holozoic species that consume particulate living and dead material.

Few studies of sediment contamination have been conducted with protozoans. Acute toxicity assays using the ciliate protozoa *Tetrahymena* sp. have only involved water exposures. Growth of *Colpidium campylum* was used to evaluate the toxicity of elutriates and sediment slurries.[1,102] Protozoan colonization of artificial substrates (polyurethane foam) were used in laboratory tests with elutriates and in situ tests with substrates suspended over contaminated sediments.[103] Community structure and functional endpoints reported by Henebry and Ross[103] include decolonization, protozoan abundance, taxa number, phototroph and heterotroph abundance, respiration, and island-epicenter colonization rates. Functional endpoints and phototrophs were the most sensitive endpoints. Stimulatory and inhibitory results were observed, and careful interpretation of effects was required.[103]

Periphyton

Benthic-associated algae (periphyton) dominate primary production in many streams[104] and shallow-lake regions.[105] Attached algal (periphyton) communities are useful indicators of aquatic pollution,[106,107] but are infrequently included in sediment studies. Shifts in community structure from pollution-sensitive groups to tolerant groups occurred in streams receiving waterborne metal[106,107] and organic pollution.[108] A continuous-flow in situ periphyton bioassay was described that measured nutrient limitation using chlorophyll and $^{14}CO_2$ uptake.[109] Outdoor experimental stream periphyton communities were sensitive to µg/L levels of pentachlorophenol, based on periphyton biomass and pigment production.[110]

Nematodes

As with the preceding biological groups, most nematode testing has been conducted on water, water-extract, or elutriate phases. Little is known about free-living freshwater nematodes. Many species are cosmopolitan in nature, can survive in a wide variety of conditions, and are primarily in the meiobenthos. They may reach densities of 100,000/m^2 and up to a depth of 2 cm in soft sediments. Nematodes can survive anoxic conditions for several weeks and have highly resistant eggs.[4] Sediment extracts and elutriate toxicity were evaluated using *Panagrellus redivivus* in 4-day exposures.[111,112] Survival, growth, and molting frequency were evaluated in

tests started with the second embryonic stage. A free-living nematode, *Caenorhabditis elegans*, was recently proposed as a promising test organism, based on ease of culture and sensitivity to metals.[113]

Oligochaetes

Oligochaetes have primarily been tested in whole-sediment exposures. Oligochaetes are a major component of benthic systems in many aquatic systems[114] and transport deeper sediments to the surface as fecal pellets. The tubificids are common in polluted areas and effectively mix sediment surface layers and play a major role in the cycling of metal and organic contaminants out of the sediments.[4,115-117] The "aquatic earthworms" used for freshwater sediment toxicity assessments are limited primarily to *Tubifex* sp. *Tubifex tubifex* is considered an indicator of organic pollution, particularly in waters with low dissolved-oxygen saturation. *Lumbriculus* has been used in whole-sediment tests to a limited extent,[118] as have some other species in Sweden.[60] However, the usefulness of oligochaetes as sediment toxicity indicators has received mixed reviews.[60,119] Taxonomy, variable sensitivity, and fragility make oligochaetes difficult to use.[60] Growth and reproduction of five oligochaete species were followed during 0.5 to 1.5-year exposures in contaminated oligotrophic sediments, and reproduction was the most sensitive endpoint.[60] *Limnodrilus* and *Stylodrilus* burrowing avoidance was a sensitive indicator of sediment contamination.[120] *T. tubifex* and *L. hoffmeisteri* avoidance behavior was observed in copper- and zinc-spiked sediments.[121] The oligotrophic *Stylodrilus heringianus* will acclimate to sediment perturbations such as mixing. Sediment reworking rates, survival, and weight were sensitive indicators of a variety of sediment contaminants[122] and Endrin-spiked sediment.[120]

Amphipods

About 150 North American freshwater species of "scuds" or "sideswimmers" have been identified.[4] The dominant species include *Hyalella azteca, Gammarus pseudolimnaens, Gammarus fasciatus, Crangonyx gracillus*, and, in the Great Lakes, *Pontoporeia hoyi* (now *Diporeia* sp.). Amphipods are widely distributed and common in unpolluted lotic and lentic systems; however, they are less common in large rivers. *Hyalella azteca* is a common and widely distributed Talitrid amphipod inhabiting permanent lakes, ponds, and streams throughout the Nearctic and Neotropical biogeographical realms.[4,123-124] In addition, *H. azteca* is euryhaline and occurs in waters of varying salinities, from 5 g/L in the estuary Barataria Bay, Louisiana[125] and athalassic Pyramid Lake, Nevada[126] to 22 g/L in saline lakes of Canada.[127] If slowly acclimated, *H. azteca* will reportedly survive at 30 g/L.[124] They are a primary food source for fish and voracious feeders of animal, plant, and detrital material.[4] The life cycle of *H. azteca* can be divided into three stages according to Cooper[128] and Pennak:[4] (1) immature (includes instars 1 to 5), (2) juvenile (includes

instars 6 and 7), and (3) adult (includes the 8th instar and beyond). The potential number of adult instars is large, and growth is indeterminate.[129] The number of molts that may occur during the adult period is variable, but may be as high as 15 or 20.[4] DeMarch[130] reported that juvenile *Hyalella azteca* completes a life cycle in 27 days or longer, depending on temperature. *H. azteca* exhibits sexual dimorphism; the adult male is larger than females and has an enlarged propodus on the second gnathopod. Eggs in the female are visible in both the ovaries and brood pouch. The epibenthic species *H. azteca*, has been used frequently in sediment toxicity testing.[28] *Hyalella azteca* has many desirable characteristics of a toxicity test organism: short generation time; easily collected from natural sources or cultured in the laboratory;[28,32,128,130,131] and data on survival, growth, and development can be obtained in toxicity tests.[28,132,133] *H. azteca* is successfully used in sediment toxicity testing and is a sensitive indicator of the presence of contaminants associated with sediments.[28,32,39,131,134-136] The amphipod juvenile stage is more sensitive to sediment contamination than the adult. *Hyalella* are easily cultured,[6,28,31,32] and standard sediment toxicity test methods were recently developed for *H. azteca* whole-sediment testing.[24,31] *Hyalella azteca* and *Gammarus* sp. have been used frequently in acute toxicity studies of pure compounds or ambient waters and found to be relatively sensitive in comparative studies.[28,34] *Pontoporeia* sp. have been used in Great Lakes studies, since it is a primary benthic species there;[16,17] unfortunately, culturing methods have not been developed; thus, deepwater collections must be made for testing.

Sediment testing with *H. azteca* has consisted primarily of whole-sediment exposures (1:4 ratios of sediment to water) in static renewal systems for 7-, 10-, 14-, 28-, or 29-day periods.[3,28,31,32,38,136] Survival is most frequently used as the endpoint in studies; however, in a 28-day chronic exposure, growth and reproductive maturation are measured.[28] *Hyalella azteca* large juveniles-young adults and *Gammarus lacustris* adults were less sensitive than *D. magna* or *C. tentans* to Cu in sediment-spiked sediment 10-day exposures.[39] *Hyalella azteca* was more sensitive than *D. magna* to Cd in static-spiked sediment tests, and only free Cd contributed to toxicity. No toxicity was observed in flow-through tests;[135] however, the flow rate was 60 volume additions per day. *Hyalella azteca* were one of the most sensitive and discriminatory of 20 different sediment toxicity assays in studies of three contaminated Great Lakes areas during 7 to 14-day whole-sediment exposures[34] and has been recommended as a tool to measure acute[32] and chronic sediment toxicity.[3,28]

Pelecypods

Mussels have been used, to a limited extent, for environmental assessments[137] and only recently in studies of sediment toxicity.[138] Bivalve mollusks are common in large rivers and vary in size from 2 to 250 mm in length. Their primary food is fine organic detritus that has been resuspended;[4] the significance of plankton as a food varies with the species and habitat. Particles as small as 1 μm can be removed by

mollusks from water. Some species burrow during their life cycle, well below the sediment surface (up to 25 cm) and have an interstitial water suspension-feeding mechanism. The life cycle of mollusks ranges from 1 month to 3 years, and they are a common food of fish, reptiles, amphibians, and mammals. The filtration capacity of mollusks is massive. An estimated 7 billion clams inhabit Lake St. Clair and theoretically filter the entire lake every 13 days, assuming each filters 4 L/day.[115] This has profound implications on their role in ecosystem dynamics. A drastic decline in species and population numbers of this ecologically and economically important group has been observed over the past three decades.[4] Recently, mollusks have been used in surveys of aquatic toxicity, both in the laboratory and in situ.[139,140] Preference-avoidance tests with *Acroneuria* indicated increased drift and locomotor activity with exposure to insecticides.[141] Celluolytic activity was sensitive to effluent toxicity in laboratory and field experiments with caged individuals.[142] Longer exposures were necessary in the laboratory to elicit response levels observed in situ. Mollusks have also been useful in long-term field monitoring studies,[32] and growth in situ seems to be a sensitive endpoint.[140] Keller and Zam[143] reported a simplified method for in vitro culturing of *Anodonta*, *Lampsilis*, and *Villosa* sp.

Insects

The mayfly (Ephemeroptera), *Hexagenia limbata*, and midges (Diptera), including *Chironomus tentans* and *C. riparius*, are the aquatic insects primarily used in sediment toxicity testing. For *H. limbata*, the sediment-dwelling nymph life stage may last from 1 to 2 years.[5] Mayflies are collectors-gatherers, with possibly some filtering at the mouth of their burrow, and have a wide distribution.[4] Midge larvae often inhabit eutrophic lakes and streams. In lotic and lentic habitats with soft sediments, about 95% of the chironomid larvae occur in the upper 10 cm of substrate.[31] The life cycle of *C. riparius* and *C. tentans* consists of three distinct stages: (1) a larval stage consisting of four instars, (2) a pupal stage, and (3) an adult stage.[31]

Hexagenia limbata has been used since the late 1970s in sediment toxicity evaluations and is sensitive to the presence of toxicants, both in laboratory and field surveys.[36,52,53] Most toxicity testing has used field-collected organisms because mayflies are difficult to culture and may only reproduce once a year. Toxicity tests have been conducted with water, interstitial water, elutriate, artificial burrows, and whole-sediment systems, using both static, static renewal, and recirculating 10-day exposures.[36,52,146,147] The measured endpoints have included mortality, molting, growth, and avoidance. Mayflies are reportedly more sensitive than other simultaneously tested species (such as *C. tentans*, *P. promelas*, *Asellus*),[10] and their response has been correlated to other species.[36] Responses have also been representative of contaminant concentrations in the sediment extracts, whole sediments, and with in situ community profiles.[18,36,145] A failure of acute responses in the laboratory to correlate with effects of benthic communities in contaminated

areas was attributed to comparing acute (10-day exposures) with in situ chronic effects.[52,53] *Hexagenia* may be less sensitive than *D. magna*,[146] but sensitivity was increased with increased exposure time (5 to 10 days).[52,53] The burrowing behavior of mayflies alters Eh, pH, organic carbon, and contaminant profiles[147] and may affect overlying water toxicant concentrations and toxicity to zooplankton.[146] The International Joint Commission (IJC) recommends their use in sediment evaluations with 14-day exposures at 20°C.[3]

Whole-sediment testing with *Chironomus* sp. was first reported by Wentsel et al.[11,12] Unlike *H. limbata*, many midge species can be easily cultured in the laboratory.[6,28,32] *Chironomus* sp. have been widely used in assays with water, interstitial water, elutriate, and whole sediment, ranging from 48-hr to 29-day exposures. Midges were generally perceived to be relatively insensitive organisms in toxicity testing. This conclusion was based on the practice of conducting short-term tests with fourth instar larvae, a procedure that may underestimate midge sensitivity to toxicants.[32,149] Midge exposures started with older larvae may underestimate midge sensitivity to toxicants. For instance, first instar *C. tentans* larvae were 6 to 27 times more sensitive than fourth instar larvae to acute copper exposure,[32,148,149] and first instar *C. riparius* larvae were 127 times more sensitive than second instar larvae to acute cadmium exposure.[148]

Chironomus sp. has been recommended as a routine whole-sediment[32] and interstitial water[49] toxicity test species. Standard sediment toxicity test methods are available for *C. tentans* and *C. riparius*.[31] The most common endpoints include mortality and growth (dry weight).[32,36] The IJC recommends growth and emergence of *C. tentans* beginning with a 13-day-old organism and continuing for 10 days or emergence.[3] Nebeker et al.[32] recommends beginning with 10-day-old organisms and continuing the assay for 15 days. Growth and survival are typical endpoints measured in chronic midge toxicity tests.[28] Wentsel measured *C. tentans* growth (length) with early instars in 17-day tests and found responses were related to bulk metal concentrations.[11,12] Emergence of mature larvae was also related to metal contamination.[15] Emergence of adult midges is a sensitive indicator of contaminant stress,[150] but is seldom monitored in toxicity tests and may be related to the choice of test species. For instance, the survival of *Chironomus tentans* in chronic toxicity tests typically exceeds 80% when exposures continue to the fourth instar; however, many of these larvae fail to pupate and emerge as adults.[150] In contrast, adult emergence by both *C. riparius* and *C. plumosus* typically exceeds 80% in chronic toxicity tests.[151]

Ingersoll and Nelson[28] observed a growth of mold and bacteria on the surface of sediment in adult emergence studies with *C. riparius*. When feeding levels were reduced enough to eliminate visible mold-bacterial growth on the surface of the sediment, larval survival was not affected, but the emergence of adults was delayed beyond 30 days, because of the dependence of adult emergence on feeding and the problem of mold-bacterial growth at higher feeding levels. Ingersoll and Nelson[28] recommend conducting *C. riparius* whole-sediment tests for 14 days, using flow-

through exposures. In this period, the first instar larvae develop to the fourth instar at 20°C, and larval survival, growth, and development can be monitored as toxicity endpoints.

In chronic toxicity tests started with first instar animals, midges were often as sensitive as daphnids to inorganic and organic compounds.[28] Sublethal response (growth) of *C. tentans* correlated with the response of Microtox®, *H. limbata*, *D. magna*, benthic community structure, and discriminated areas of contamination.[36,89] In recent comparative testing, *C. riparius* was more sensitive than *C. tentans*, in several contaminated whole-sediment assays.[34]

CONCLUSIONS

Benthic organisms, as a group, are the best overall indicators of toxic sediments due to (1) their direct contact with sediment solids and interstitial waters; (2) our knowledge of the relative pollution sensitivity and life histories of many species; and (3) the proven effectiveness of amphipod, midge, and mayfly larvae assays in detecting sediment toxicity in a wide range of studies. Unfortunately, the most commonly used assays (e.g., *H. azteca* 10-day survival, *C. tentans* 10-day survival and growth, *H. limbata* 10-day survival) are not ideal toxicity indicators. Problems such as laboratory culturing and recovery of early instar organisms can and will be relatively difficult to overcome. Reliable, efficient, and sensitive chronic toxicity assays have not been widely reported, but represent an active area of current research. In the interim period it seems prudent to include more conventional chronic toxicity indicators, such as *Pimephales promelas* early life stage and *Ceriodaphnia dubia* three-brood reproduction and survival assays, in sediment toxicity test batteries that also include benthic indicators. In the near future our understanding of sublethal indicators (biomarkers) and of the relationship between acute and chronic effects may allow relatively short-term exposures, e.g., hours to several days, to be reliably used in estimates of chronic effects. Presently, they represent a key component of integrated ecosystem health assessments.

REFERENCES

1. Munawar, M., I. F. Munawar, C. I. Mayfield, and L. H. McCarthy. "Probing Ecosystem Health: A Multi-Disciplinary and Multi-Trophic Assay Strategy," *Hydrobiologia* 188/189:93–116 (1989).
2. Great Lakes Science Advisory Board. "Literature Review of the Effects of Persistent Toxic Substances on Great Lakes Biota," International Joint Commission, Windsor, Ontario (1986).
3. Great Lakes Water Quality Board. "Procedures for the Assessment of Contaminated Sediment Problems in the Great Lakes," International Joint Commission, Windsor, Ontario (1988).

4. Pennak, R. W. *Freshwater Invertebrates of the United States: Protozoa to Mollusca*, 3rd Ed. (New York: John Wiley & Sons, 1989).
5. Merritt, R. W., and K. W. Cummins. *An Introduction to the Aquatic Insects of North America* (Dubuque, IA: Kendall/Hunt, 1984).
6. Lawrence, S. G., Ed. *Manual for the Culture of Selected Freshwater Invertebrates*. Can. Spec. Publ. Fish. Aquat. Sci. 54:169 (1981).
7. Cummins, K. W. "Structure and Function of Stream Ecosystems," *Bioscience* 24:631–641 (1974).
8. Carpenter, S. R. *Complex Interactions in Lake Communities* (New York: Springer-Verlag, 1988).
9. Minshall, G. W. "Stream Ecosystem Theory: A Global Perspective." *J. N. Am. Benthol. Soc.* 7:263–288 (1988).
10. Prater, B. L., and M. A. Anderson. "A 96-h Bioassay of Duluth and Superior Harbor Basins (Minnesota) Using *Hexagenia limbata*, *Asellus communis*, *Daphnia magna*, and *Pimphales promelas* as Test Organisms," *Bull. Environ. Contam. Toxicol.* 18:159–169 (1977).
11. Wentsel, R., A. McIntosh, W. P. McCafferty, G. Atchison, and V. Anderson. "Avoidance Response of Midge Larvae *(Chironomus tentans)* to Sediments Containing Heavy Metals," *Hydrobiologia* 55:171–175 (1977).
12. Wentsel, R., A. McIntosh, and G. Atchison. "Sublethal Effects of Heavy Metal Contaminated Sediment on Midge Larvae *(Chironomus tentans)*," *Hydrobiologia* 56:153–156 (1977).
13. Prater, B., and R. A. Hoke. "A Method for the Biological and Chemical Evaluation of Sediment Toxicity," in *Contaminants and Sediments*, Vol. 1, R. A. Baker, Ed. (Ann Arbor, MI: Ann Arbor Science, 1980), pp. 483–499.
14. Laskowski-Hoke, R. A., and B. L. Prater. "Dredged Material Evaluation: Correlations Between Chemical and Biological Procedures," *J. Water Pollut. Contr. Fed.* 53:1260–1262 (1981).
15. Wentsel, R., A. McIntosh, and W. P. McCafferty. "Emergence of the Midge *Chironomus tentans* When Exposed to Heavy Metal Contaminated Sediment," *Hydrobiologia* 57:195–196 (1978).
16. Gannon, J. E., and A. M. Beeton. "Procedures for Determining the Effects of Dredged Sediments on Biota-Benthos Viability and Sediment Selectivity Tests. *J. Water Pollut. Contr. Fed.* 43:392–398 (1971).
17. U.S. Environmental Protection Agency. *Development of Bioassay Procedures for Defining Pollution of Harbor Sediments*. U.S. EPA Environmental Research Laboratory, Duluth, MN (1981).
18. Prater, B. L., and M. A. Anderson. "A 96-Hour Bioassay of Otter Creek, Ohio," *J. Water Pollut. Contr. Fed.* 49:2099–2106 (1977).
19. Peddicord, R. K. "Direct Effects of Suspended Sediments on Aquatic Organisms," in *Contaminants and Sediments*, Vol. 1, R. A. Baker, Ed. (Ann Arbor, MI: Ann Arbor Science, 1980), pp. 501–536.
20. U. S. Environmental Protection Agency, U.S. Army Corps of Engineers. *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters*. Environmental Effects Laboratory, Experimental Waterways Experiment Station, Vicksburg, MI (1977).

21. Lee, G. F., and R. A. Jones. "Water Quality Significance of Contaminants Associated With Sediments: An Overview," in *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*, K. L. Dickson, A. W. Maki, and W. A. Brungs, Eds. (New York: Pergamon Press, 1984), pp. 1–34.
22. Anderson, J., W. Birge, J. Gentile, J. Lake, J. Rodgers, Jr., and R. Swartz. "Biological Effects, Bioaccumulation, and Ecotoxicology of Sediment-Associated Chemicals," in *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*, K. L. Dickson, A. W. Maki, and W. A. Brungs, Eds. (New York: Pergamon Press, 1984) pp. 267–296.
23. Burton, G. A., Jr., Ed. *Sediment Toxicity Assessment*. (Boca Raton, FL: Lewis Publishers, 1992).
24. Ingersoll, C. "Sediment Toxicity and Bioaccumulation Testing," American Society for Testing and Materials, *ASTM Standardization News*, (pp. 29–33, April 1991).
25. Burton, G. A., Jr., J. M. Lazorchak, W. T. Waller, and G. R. Lanza. "Arsenic Toxicity Changes in the Presence of Sediment," *Bull. Environ. Contam. Toxicol.* 38:491–499 (1987).
26. David, W. S., L. A. Fay, and C. E. Herdendorf. "Overview of USEPA/Clear Lake Erie Sediment Oxygen Demand Investigations During 1979," *J. Great Lakes Res.* 13:731–737 (1987).
27. Francis, P. C., W. J. Birge, and J. A. Black. "Effects of Cadmium-Enriched Sediment on Fish and Amphibian Embryo-Larval Stages. *Ecotoxicol. Environ. Saf.* 8:378–387 (1987).
28. Ingersoll, C., and M. K. Nelson. "Testing Sediment Toxicity With *Hyalella azteca* (Amphipoda) and *Chironomus riparius* (Diptera)," in *Aquatic Toxicology and Risk Assessment: Thirteenth Volume*, STP 1096, W. G. Landis, and W. H Vander Schalie, Eds., (Philadelphia: American Society of Testing and Materials, 1990), pp. 93–109.
29. Di Toro, D. M., J. D. Mahony, D. J. Hansen, K. J. Scott, M. B. Hicks, S. M. Mayr, and M. S. Redmond. "Toxicity of Cadmium in Sediments: The Role of Acid Volatile Sulfide," *Environ. Toxicol. Chem.* 9:1487–1502 (1990).
30. Schuytema, G. S., P. O. Nelson, K. W. Malueg, A. G. Nebeker, D. F. Krawczyk, A. K. Ratcliff, and J. H. Gakstatter. "Toxicity of Cadmium in Water and Sediment Slurries to *Daphnia magna*. *Environ. Toxicol. Chem.* 3:293–308 (1984).
31. American Society for Testing and Materials. *Standard Guide for Conducting Toxicity Tests with Freshwater Invertebrates*, (Philadelphia: American Society for Testing and Materials, 1990), Standard E (in press).
32. Nebeker, A. V., M. A. Cairns, J. H. Gakstatter, K. W. Malueg, G. S. Schuytema, and D. F. Krawczyk. "Biological Methods for Determining Toxicity of Contaminated Freshwater Sediments to Invertebrates," *Environ. Toxicol. Chem.* 3:617–630 (1984).
33. Stemmer, B. L., G. A. Burton, Jr., and G. Sasson-Brickson. "Effect of Sediment Spatial Variance and Collection Method on Cladoceran Toxicity and Indigenous Microbial Activity Determinations," *Environ. Toxicol. Chem.* 9:1035–1044 (1990).
34. Burton, G. A., Jr., L. Burnett, M. Henry, S. Klaine, P. Landrum, and M. Swift. "A Multi-Assay Comparison of Sediment Toxicity at Three 'Areas of Concern'," Abstr. Annu. Meet Soc. Environ. Toxicol. Chem., Arlington, VA No. 213, p. 53, (1990).

35. Sasson-Brickson, G., and G. A. Burton, Jr. "*In Situ* and Laboratory Sediment Toxicity Testing With *Ceriodaphnia dubia*," *Environ. Toxicol. Chem.* 10:201–207 (1991).
36. Giesy, J. P., C. J. Rosieu, R. L. Graney, and M. G. Henry. "Benthic Invertebrate Bioassays With Toxic Sediment and Pore Water," *Environ. Toxicol. Chem.* 9:233–248 (1990).
37. Hoke, R. A., J. P. Giesy, G. T. Ankley, J. L. Newsted, and J. R. Adams. "Toxicity of Sediments from Western Lake Erie and the Maumee River at Toledo, Ohio, 1987: Implications for Current Dredged Material Disposal Practices," *J. Great Lakes Res.* 16:457–470 (1990).
38. Burton, G. A., Jr., B. L. Stemmer, and K. L. Winks. "A Multitrophic Level Evaluation of Sediment Toxicity in Waukegan and Indiana Harbors," *Environ. Toxicol. Chem.* 8:1057–1066 (1989).
39. Cairns, M. A., A. V. Nebeker, J. N. Gakstatter, and W. L. Griffis. "Toxicity of Copper-Spiked Sediments to Freshwater Invertebrates," *Environ. Toxicol. Chem.* 3:435–445 (1984).
40. Ankley, G. T., A. Katko, and J. W. Arthur. "Identification of Ammonia as in Important Sediment-Associated Toxicant in the Lower Fox River and Green Bay, Wisconsin," *Environ. Toxicol. Chem.* 9:313–322 (1990).
41. Brannon, J. M., R. H. Plumb, Jr., and I. Smith, Jr. "Long-Term Release of Heavy Metals for Sediments, in *Contaminants and Sediments*, Vol. 1, R.A. Baker, Ed., (Ann Arbor, MI: Ann Arbor Science, 1980) pp. 221–266.
42. Lamberson, J., and R. Swartz. "Use of Bioassays in Determining the Toxicity of Sediment to Benthic Organisms," in *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus*, M. S. Evans, Ed. (New York: John Wiley & Sons, 1988), pp. 257–279.
43. Pittinger, C. A., V. C. Hand, J. A. Masters, and L. F. Davidson. "Interstitial Water Sampling in Ecotoxicological Testing: Partitioning of a Cationic Surfactant," in *Aquatic Toxicology and Hazard Assessment: 10th Volume*, STP 971, W. J. Adams, G. A. Chapman, and W. G. Lundis, Eds. (Philadelphia: American Society for Testing and Materials, 1988), pp. 138–148.
44. American Society for Testing and Materials. *Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing*, ASTM Standard No. E1391 (Philadelphia: American Society for Testing and Materials, 1991).
45. Knezovich, J. P., F. L. Harrison, and R. G. Wilhelm. "The Bioavailability of Sediment-Sorbed Organic Chemicals: A Review," *Water, Air, Soil Pollut.* 32:233–245 (1987).
46. Adams, W. J., R. A. Kimerle, and R. G. Mosher. "Aquatic Safety Assessment of Chemicals Sorbed to Sediments," in *Aquatic Toxicology and Hazard Assessment*, Seventh Symposium, STP 854 (Philadelphia: American Society for Testing and Materials, 1985), pp. 429–453.
47. Eadie, B. J., P. F. Landrum, and W. Faust. "Polycyclic Aromatic Hydrocarbons in Sediments, Pore Water and the Amphipod *Pontoporeia hoyi* from Lake Michigan," *Chemosphere* 11:847–858 (1982).
48. Spehar, R. L., R. L. Anderson, and J. T. Fiandt. "Toxicity and Bioaccumulation of Cadmium and Lead in Aquatic Invertebrates," *Environ. Pollut.* 15:195–208 (1978).

49. Giesy, J. P., and R. A. Hoke. "Freshwater Sediment Toxicity Bioassessment: Rationale for Species Selection and Test Design," *J. Great Lakes Res.* 15:539–569 (1989).
50. Birge, W. J., J. A. Black, A. G. Westerman, and P. C. Francis. "Toxicity of Sediment-Associated Metals to Freshwater Organisms: Biomonitoring Procedures," in *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*, K. L. Dickson, A. W. Maki, and W. A. Brungs, Eds. (New York: Pergamon Press, 1984) pp. 199–218.
51. LeBlanc, G. A., and D. C. Surprenant. "A Method of Assessment the Toxicity of Contaminated Freshwater Sediments," in *Aquatic Toxicology and Hazard Assessment*, Seventh Symposium, STP 854, R. D. Cardwell, R. Purdy, and R. C. Bahner, Eds. (Philadelphia: American Society of Testing and Materials, 1985), pp. 269–283.
52. Malueg, K. W., G. S. Schuytema, D. F. Krawczyk, and J. H. Gakstatter. "Laboratory Sediment Toxicity Tests, Sediment Chemistry and Distribution of Benthic Macroinvertebrates in Sediments from the Keweenaw Waterway, Michigan," *Environ. Toxicol. Chem.* 3:233–242 (1984).
53. Malueg, K. W., G. S. Schuytema, J. H. Gakstatter, and D. F. Krawczyk. "Toxicity of Sediments from Three Metal-Contaminated Areas," *Environ. Toxicol. Chem.* 3:279–291 (1984).
54. Chapman, P. M. "A Bioassay by Another Name Might Not Smell the Same," *Environ. Toxicol. Chem.* 8:551 (1989).
55. Suter, G. W. "Seven-Day Tests and Chronic Tests," *Environ. Toxicol. Chem.* 9:1435–1436 (1990).
56. Norberg-King, T. J. "An Evaluation of the Fathead Minnow Seven-Day Subchronic Test for Estimating Chronic Toxicity," *Environ. Toxicol. Chem.* 8:1075–1089 (1984).
57. Giesy, J. P., and R. L. Grancy. "Recent Developments in and Intercomparisons of Acute and Chronic Bioassays and Bioindicators," *Hydrobiologia* 188/189:21–60 (1989).
58. Winner, R. "Multigeneration Life-Span Tests of the Nutritional Adequacy of Several Diets and Culture Waters for *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* 8:513–520 (1988).
59. Rand, G. M., and S. R. Petrocelli. *Fundamentals of Aquatic Toxicology*. (New York: Hemisphere, 1985).
60. Wiederholm, T., A. Wiederholm, and G. Milbrink. "Bulk Sediment Bioassays with Five Species of Fresh-Water Oligochaetes," *Water, Air Soil Pollut.* 36:131–154 (1987).
61. Swartz, R., W. A. DeBen, J. K. Jones, J. O. Lamberson, and F. A. Cole. "Phoxocephalid Amphipod Bioassay for Hazard Assessment," in *Aquatic Toxicology and Hazard Assessment: Proceedings of the Seventh Annual Symposium*, STP 854, R. D. Cardwell, R. Purdy, and R. C. Bahner, Eds. (Philadelphia: American Society for Testing and Materials, 1985), pp. 284–307.
62. U.S. Environmental Protection Agency. *Technical Support Document for Water Quality-Based Toxics Control*, Office of Water, Washington, D.C. (1985).
63. Walker, J. D. "An U.S. EPA Perspective on Ecotoxicity Testing Using Microorganisms," in *Toxicity Testing Using Microorganisms*, Vol. II, B. J. Dutka, and G. Bitton, Eds. (Boca Raton, FL, CRC Press, 1986) pp. 175–186.
64. Babich, H., and G. Stotzky. "Developing Standards for Environmental Toxicants: The Need to Consider Abiotic Environmental Factors and Microbe-mediated Ecologic Processes," *Environ. Health Perspect.* 49:247–260 (1983).
65. Odum, E. P. "Trends Expected in Stressed Ecosystems," *Bioscience* 35:419–422 (1985).

66. Griffiths, R. P. "The Importance of Measuring Microbial Enzymatic Functions While Assessing and Predicting Long-Term Anthropogenic Perturbations," *Mar. Pollut. Bull.* 14:162–165 (1983).
67. Porter, K. G., H. Paerl, R. Hodson, M. Pace, J. Priscu, B. Riemann, D. Scavia, and J. Stockner. "Microbial Interactions in Lake Food Webs," in *Complex Interactions in Lake Communities*, S. R. Carpenter, Ed. (New York: Springer-Verlag, 1987), pp. 209–227.
68. Burton, G. A., Jr., and B. L. Stemmer. "Evaluation of Surrogate Tests in Toxicant Impact Assessments," *Tox. Assess.* 3:255–269 (1988).
69. Stebbing, A. R. D. "Hormesis — The Stimulation of Growth by Low Levels of Inhibitors," *Sci. Tot. Environ.* 22:213–234 (1982).
70. Calabrese, E. J., and M. McCarthy. "Hormesis: A New Challenge to Current Approaches for Estimating Cancer Risks Associated with Low Doses," *Water Res. Q.* 4:12–15 (1986).
71. Pratt, J. R., N. J. Bowers, B. R. Niederlehner, and J. Cairns, Jr. "Effects of Atrazine on Freshwater Microbial Communities," *Arch. Environ. Contam. Toxicol.* 17:449–457 (1988).
72. Griffiths, R. P., B. A. Caldwell, W. A. Broich, and R. Y. Morita. "Long-Term Effects of Crude Oil on Microbial Processes in Subarctic Marine Sediments," *Mar. Pollut. Bull.* 13:273–278 (1982).
73. Schiewe, M. H., E. G. Hawk, D. I. Actor, and M. M. Krahn. "Use of Bacterial Bioluminescence Assay to Assess Toxicity of Contaminated Marine Sediments," *Can. J. Fish. Aquat. Sci.* 42:1244–1248 (1985).
74. Dutka, B. J. "Method for Determining Acute Toxicant Activity in Water, Effluents and Leachates Using *Spirillum volutans*," *Tox. Assess.* 1:139–145 (1986).
75. Palmateer, G. A., D. E. McLean, M. J. Walsh, W. L. Kutus, E. M. Janzen, and D. E. Hocking. "A Study of Contamination of Suspended Stream Sediments With *Escherichia coli*," *Tox. Assess.* 4:377–397 (1989).
76. Williamson, K. S., and D. G. Johnson. "A Bacterial Bioassay for Assessment of Wastewater Toxicity," *Water Res.* 15:383 (1981).
77. Tam, T.-Y., and J. T. Trevors. "Toxicity of pentachlorophenol to *Azobacter vinelandii*," *Bull. Environ. Contam. Toxicol.* 27:230 (1981).
78. Flemming, C. A., and J. T. Trevors. "Copper Toxicity in Freshwater Sediment and *Aeromonas hydrophila* Cell Suspensions Measured Using an O_2 Electrode," *Tox. Assess.* 4:473–485 (1989).
79. Trevors, J. T., C. I. Mayfield, and W. E. Inniss. "A Rapid Toxicity Test Using *Pseudomonas fluorescens*," *Bull. Enviorn. Contam. Toxicol.* 26:433 (1981).
80. De Zwart, D., and W. Sloof. "The Microtox as an Alternative Assay in the Acute Toxicity Assessment of Water Pollutants," *Aquat. Toxicol.* 4:129 (1983).
81. Dutka, B. J., K. Jones, K. K. Kwan, H. Bailey, and R. McInnis. "Use of Microbial and Toxicant Screening Tests for Priority Size Selection of Degraded Areas in Water Bodies," *Water Res.* 22:503–510 (1988).
82. Babich, H., and G. Stotzky. "Heavy Metal Toxicity to Microbe-Mediated Ecologic Processes: A Review and Potential Application to Regulatory Policies," *Environ. Res.* 36:111–137 (1985).
83. Bihari, N., M. Najdek, R. Floris, R. Batel, and R. K. Zahn. "Sediment Toxicity Assessment Using Bacterial Bioluminescence: Effect of an Unusual Phytoplankton Bloom," *Mar. Ecol. Prog. Ser.* 57:307–310 (1989).

84. Hinwood, A. L., and M. J. McCormick. "The Effect of Ionic Strength of Solutes on EC_{50} Values Measured During the Microtox Test," *Tox. Assess.* 2:449–461 (1987).
85. True, C. J., and A. A. Heyward. "Relationships Between Microtox Test Results, Extraction Methods, and Physical and Chemical Compositions of Marine Sediment Samples," *Tox. Assess.* 5:29–45 (1990).
86. Bulich, A. A. "Bioluminescence Assays," in *Toxicity Testing Using Microorganisms*, Vol. 1, G. Bitton, and B. J. Dutka, Eds. (Boca Raton, FL, CRC Press, 1986) pp. 57–74.
87. Greene, J. C., W. E. Miller, M. K. Debacon, M. A. Long, and C. L. Bartels. "A Comparison of Three Microbial Assay Procedures for Measuring Toxicity to Chemical Residues," *Arch. Environ. Contam. Toxicol.* 14:657–667 (1985).
88. Dutka, B. J., and K. K. Kwan. "Studies on a Synthetic Activated Sludge Toxicity Screening Procedure with Comparison to Three Microbial Toxicity Tests," in *Toxicity Screening Procedures Using Bacterial Systems*, B. J. Dutka, and D. Liu, Eds. (New York: Marcel Dekker, 1984), pp. 125–138.
89. Giesy, J. P., R. L. Graney, J. L. Newsted, C. J. Rosiu, A. Benda, R. G. Kreis, Jr., and F. J. Horvath. "Comparison of Three Sediment Bioassay Methods Using Detroit River Sediments," *Environ. Toxicol. Chem.* 7:483–498 (1988).
90. Athey, L. A., J. M. Thomas, W. E. Miller, and J. Q. Word. "Evaluation of Bioassays for Designing Sediment Cleanup Strategies at a Wood Treatment Site," *Environ. Toxicol. Chem.* 8:223–230 (1989).
91. Brouwer, H., T. Murphy, and L. McArdle. "A Sediment-Contact Bioassay With *Photobacterium phosphoreum*," *Environ. Toxicol. Chem.* 9:1353–1358 (1990).
92. Capone, D. G., D. D. Reese, and R. P. Kline. "Effects of Metals on Methanogenesis, Sulfate Reduction, Carbon Dioxide Evolution, and Microbial Biomass in Anoxic Salt Marsh Sediments," *Appl. Environ. Microbiol.* 45:1586–1591 (1983).
93. Furutani, A., and J. W. M. Rudd. "A Method for Measuring the Response of Sediment Microbial Communities to Environmental Perturbations," *Can. J. Microbiol.* 30:1408–1414 (1984).
94. Baker, J. H., and R. Y. Morita. "A Note on the Effects of Crude Oil on Microbial Activities in Stream Sediment," *Environ. Pollut.* 31:149–157 (1983).
95. Buikema, A. L., C. L. Rutherford, and J. Cairns, Jr. "Screening Sediments for Potential Toxicity by In Vitro Enzyme Inhibition," in *Contaminants and Sediments*, Vol. 1, R. A. Baker, Ed. (Ann Arbor, MI: Ann Arbor Science, 1980), pp. 463–475.
96. Sayler, G. S., M. Puziss, and M. Silver. "Alkaline Phosphatase Assay for Freshwater Sediments: Application to Perturbed Sediment Systems," *Appl. Environ. Microbiol.* 38:922–927 (1979).
97. Sayler, G. S., T. W. Sherrill, R. E. Perkins, L. M. Mallory, M. P. Shiaris, and D. Pedersens. "Impact of Coal-Coking Effluent on Sediment Microbial Communities: A Multivariate Approach," *Appl. Environ. Microbiol.* 44:1118–1129 (1982).
98. Sayler, G. S., R. E. Perkins, T. W. Sherrill, B. K. Perskins, M. C. Reid, M. S. Shields, H. L. Kong, and J. W. Davis. "Microcosm and Experimental Pond Evaluation of Microbial Community Response to Synthetic Oil Contamination in Freshwater Sediments," *Appl. Environ. Microbiol.* 46:211–219 (1983).

99. Heitkamp, M. A., and B. T. Johnson. "Impact of an Oil Field Effluent on Microbial Activities in a Wyoming River," *Can. J. Microbiol.* 30:786–792 (1984).
100. Pratt, J. R., and J. Cairns. "Functional Groups in the Protozoa: Roles in Differing Ecosystems," *J. Protozool.* 32:415–423 (1985).
101. Picken, L. E. R. "The Structure of Some Protozoan Communities," *J. Ecol.* 25:368–384 (1937).
102. Dive, D., S. Robert, E. Angrand, C. Bel, H. Bonnemain, L. Brun, Y. Demarque, A. Le Du, R. El Bouhouti, M. N. Fourmaux, L. Guery, O. Hanssens, and M. Murat. "A Bioassay Using the Measurement of the Growth Inhibition of a Ciliate Protozoan: *Colpidium campylum* Stokes," in *Environmental Bioassay Techniques and Their Application*, M. Munawar, G. Dixon, C. I. Mayfield, T. Reynoldson, and H. Sadar, Eds., *Hydrobiologia* 188/189: 181–188 (1989).
103. Henebry, M. S., and P. E. Ross. "Use of Protozoan Communities to Assess the Ecotoxicological Hazard of Contaminated Sediments," *Tox. Assess.* 4:209–227 (1989).
104. Bott, T. L., and L. A. Kaplan. "Bacterial Biomass, Metabolic State, and Activity in Stream Sediments: Relation to Environmental Variables and Multiple assay Comparisons," *Appl. Environ. Microbiol.* 50:508–522 (1985).
105. Carlton, R. G., and M. J. Klug. "Spatial and Temporal Variations in Microbial Processes in Aquatic Sediments: Implications for the Nutrient Status of Lakes," in *Sediments: Chemistry and Toxicity of In-Place Pollutants*, R. Baudo, J. Giesy,, and H. Muntau, Eds. (Boca Raton, FL: Lewis Publishers, 1990), pp. 107–130.
106. Burton, G. A., Jr., A. Drotar, J. M. Lazorchak, and L. L. Bahls. "Relationship of Microbial Activity and *Ceriodaphnia* Responses to Mining Impacts on the Clark Fork River, Montana," *Arch. Environ. Contam. Toxicol.* 16:523–530 (1987).
107. Genter, R. B., D. S. Cherry, E. P. Smith, and J. C. Cairns, Jr. "Attached-Algal Abundance Altered by Individual and Combined Treatments of Zinc and pH," *Environ. Toxicol. Chem.* 7:723–733 (1988).
108. Lange-Bertalot, H. "Pollution Tolerance of Diatoms as a Criterion for Water Quality Estimation," *Nova Hedwigia, Beiheft* 64:285–304 (1979).
109. Peterson, B. J., J. E. Hobbie, T. L. Corliss, and K. Kriet. "A Continuous-Flow Periphyton Bioassay: Tests of Nutrient Limitation in a Tundra Stream," *Limnol. Oceanogr.* 28:583–591 (1983).
110. Yount, J. D., and J. E. Richter. "Effects of Pentachlorophenol on Periphyton Communities in Outdoor Experimental Streams," *Arch. Environ. Contam. Toxicol.* 15:51–60 (1986).
111. Samoiloff, M. R., J. Bell, D. A. Birkholz, G. R. Webster, E. G. Arnott, R. Pulak, and A. Madrid. Combined Bioassay-Chemical Fraction Scheme for the Determination and Ranking of Toxic Chemicals in Sediments," *Environ. Sci. Technol.* 17:329–334 (1983).
112. Ross, P. E., L. C. Burnett, and M. S. Henebry. "Chemical and Toxicological Analyses of Lake Calumet (Cook County, Illinois) Sediments," Report No. HWRIC RR-036, Illinois Hazardous Waste Research and Information Center, Champaign, IL (1989).
113. Williams, P. L., and D. B. Dusenbery. "Aquatic Toxicity Testing Using the Nematode, *Caenorhabditis elegans*," *Environ. Toxicol. Chem.* 9:1285–1290 (1990).
114. Brinkhurst, R. O. *The Benthos of Lakes* (London: Macmillan Press, 1974).

115. Reynoldson, T. B. "Interactions Between Sediment Contaminants and Benthic Organisms," *Hydrobiologia* 149:53–66 (1987).
116. Karickhoff, S. W., and K. R. Morris. "Impact of Tubificid Oligochaetes on Pollutant Transport in Bottom Sediments," *Environ. Sci. Technol.* 19:51–56 (1985).
117. Jernelöv, A. "Release of Methyl Mercury from Sediments with Layers Containing Inorganic Mercury at Different Depths," *Limnol. Oceanogr.* 15:958–960 (1970).
118. Bailey, H. C., and D. H. Liu. "*Lumbriculus variegatus*, a Benthic Oligochaete, as a Bioassay Organism," in *Aquatic Toxicology*, STP 707, (Philadelphia: American Society of Testing and Materials, 1980), pp. 205–215.
119. Chapman, P. M., and R. O. Brinkhurst. "Lethal and Sublethal Tolerances of Aquatic Oligochaetes with Reference to Their Use as a Biotic Index of Pollution," *Hydrobiologia* 115:139–144 (1984).
120. Keilty, T. J., D. S. White, and P. F. Landrum. "Short-Term Lethality and Sediment Avoidance Assays with Endrin-Contaminated Sediment and Two Oligochaetes from Lake Michigan," *Arch. Environ. Contam. Toxicol.* 17:95–101 (1988).
121. McMurthy, M. J. "Avoidance of Sublethal Doses of Copper and Zinc by Tubificid Oligochaetes," *J. Great Lakes Res.* 10:267–272 (1984).
122. Keilty, T. J., and P. F. Landrum. "Population-Specific Toxicity Responses by the Freshwater Oligochaete, *Stylodrilus heringianus*, in Natural Lake Michigan Sediments," *Environ. Toxicol. Chem.* 9:1147–1154 (1990).
123. Bousfield, E. L. "Fresh-Water Amphipod Crustaceans of Glaciated North America," *Can. Field Nat.* 72:55–113 (1958).
124. deMarch, B. G. E. "*Hyalella azteca* (Saussure)," in *A Manual for the Culture of Selected Freshwater Invertebrates*, S. G. Lawrence, Ed., *Can. Spec. Publ. Fish. Aquat. Sci.* 54:61–78 (1981).
125. Thomas, J. D. "A Survey of Gammarid Amphipods of the Barataria Bay, Louisiana Region," *Cont. Mar. Sci.* 20:87–100 (1976).
126. Galat, D. L., M. Coleman, and R. Robinson. "Experimental Effects of Elevated Salinity on Three Benthic Invertebrates in Pyramid Lake, Nevada," *Hydrobiologia* 158:133–144 (1988).
127. Timms, B. V., U. T. Hammer, and J. W. Sheard. "A Study of Benthic Communities in Some Saline Lakes in Saskatchewan and Alberta, Canada," *Int. Rev. ges. Hydrobiol.* 71:759–777 (1986).
128. Cooper, W. E. "Dynamics and Production of a Natural Population of a Fresh-Water Amphipod, *Hyalella azteca*," *Ecol. Monogr.* 35:377–394 (1965).
129. Strong, D. R. "Life History Variation Among Populations of an Amphipod (*Hyalella azteca*)," *Ecology* 53:1103–1111 (1972).
130. deMarch, B. G. E. "The Effects of Constant and Variable Temperatures on the Size, Growth, and Reproduction of the Freshwater Amphipod *Hyalella azteca* (Saussure)," *Can. J. Zool.* 56:1801–1806 (1978).
131. Borgmann, U., and M. Munawar. "A New Standardized Sediment Bioassay Protocol Using the Amphipod *Hyalella azteca* (Saussure)," *Hydrobiologia* 188/189:425–531 (1989).
132. Maciorowski, H. D. "Comparison of the Lethality of Selected Industrial Effluents Using Various Aquatic Invertebrates Under Laboratory Conditions," Technical Report No. CEN/T-75-3, Department of the Environment, Fisheries and Marine Service, Central Region, Winnipeg, Canada (1975).

133. Borgmann, U., K. M. Ralph, and W. P. Norwood. "Toxicity Test Procedures for *Hyalella azteca*, and Chronic Toxicity of Cadmium and Pentachlorophenol to *H. azteca*, *Gammarus fasciatus*, and *Daphnia magna*," *Arch. Environ. Contam. Toxicol.* 18 (1989).
134. Landrum, P. F., and D. Scavia. "Influence of Sediment on Anthracene Uptake, Depuration, and Biotransformation by the Amphipod *Hyalella azteca*," *Can. J. Fish. Aquat. Sci.* 40:298–305 (1983).
135. Nebeker, A. V., S. T. Onjukka, M. A. Cairns, and D. F. Krawczyk. "Survival of *Daphnia magna* and *Hyalella azteca* in Cadmium-Spiked Water and Sediment," *Environ. Toxicol. Chem.* 5:933–938 (1986).
136. Nebeker, A. V., and C. E. Miller. "Use of the Amphipod Crustacean *Hyalella azteca* in Freshwater and Estuarine Sediment Toxicity Tests," *Environ. Toxicol. Chem.* 7:1027–1033 (1988).
137. Imlay, M. J. "Use of Shells of Freshwater Mussels in Monitoring Heavy Metals and Environmental Stresses: A Review," *Mamacol. Rev.* 15:1–14 (1982).
138. Wade, D. C., and R. G. Hudson. "The Use of Juvenile Freshwater Mussels as a Laboratory Test Species for Evaluating Environmental Toxicity," Abstr. Annu. Meet. Soc. Environ. Toxicol. & Chem., Toronto, Ontario, No. P232, p. 247 (1989).
139. Gentile, J. H., and K. J. Scott. "The Application of a Hazard Assessment Strategy to Sediment Testing: Issues and Case Study," in *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*, K. L. Dickson, A. W. Maki, and W. A. Brungs, Eds. (New York: Pergamon Press, 1984) pp. 167–182.
140. Dickson, K. L., W. T. Waller, J. H. Kennedy, W. R. Arnold, W. P. Desmond, S. D. Dyer, J. F. Hall, J. T. Knight, Jr., D. Malas, M. L. Martinez, and S. L. Matzner. "A Water Quality and Ecological Survey of the Trinity River. Vol. 1," City of Dallas Water Utilities, Dallas, TX (1989).
141. Scherer, E., and R. E. McNicol. "Behavioral Responses of Stream-Dwelling *Acroneuria lycorias* (Ins., Plecopt.) Larvae to Methoxychlor and Fenitrothion," *Aquat. Toxicol.* 8:251–263 (1986).
142. Farris, J. L., J. H. van Hassel, S. E. Belanger, D. S. Cherry, and J. Cairns, Jr. "Application of Cellulolytic Activity of Asiatic Clams (*Corbicula* sp.) to In-Stream Monitoring of Power Plant Effluents," *Envrion. Toxicol. Chem.* 7: 701–713 (1988).
143. Keller, A. E., and S. G. Zam. "Simplification of In Vitro Culture Techniques for Freshwater Mussels," *Environ. Toxicol. Chem.* 9:1291–1296 (1990).
144. Fremling, C. D., and W. L. Mauck. "Methods for Using Nymphs of Burrowing Mayflies (Ephemeroptera, Hexagenia) as Toxicity Test Organisms," in *Aquatic Invertebrate Bioassays*, STP 715, A. L. Buikema, Jr., and J. Cairns, Jr., Eds. (Philadelphia: American Society of Testing and Materials, 1980), pp. 81–97.
145. Hoke, R. A., and B. L. Prater. "Relationship of Percent Mortality of Four Species of Aquatic Biota from 96-Hour Sediment Bioassays of Five Lake Michigan Harbors and Elutriate Chemistry of the Sediments," *Bull. Environ. Contam. Toxicol.* 25:394–399 (1980).
146. Malueg, K. W., G. S. Schuytema, J. H. Gakstatter, and D. F. Krawczyk. "Effect of *Hexagenia* on *Daphnia* Responses in Sediment Toxicity Tests," *Environ. Toxicol. Chem.* 2:73–82 (1983).
147. Gerould, S., and S. P. Gloss. "Mayfly-Mediated Sorption of Toxicants into Sediments," *Environ. Toxicol. Chem.* 5:667–673 (1980).

148. Gauss, J. D., P. E. Woods, R. W. Winner, and J. H. Skillings. "Acute Toxicity of Copper to Three Life Stages of *Chironomus tentans* as Affected by Water Hardness-Alkalinity," *Environ. Bull. (Ser. A)* 37:149–157 (1985).
149. Williams, K. A., D. W. J. Green, D. Pascoe, and D. E. Gower. "The Acute Toxicity of Cadmium to Different Larval Stages of *Chironomus riparius* (Diptera: Chironomidae) and Its Ecological Significance for Pollution Regulation," *Oecologia* 70:362–366 (1986).
150. Nebeker, A. V., M. A. Cairns, and C. M. Wise. "Relative Sensitivity *Chironomus tentans* Life Stages to Copper," *Environ. Toxicol. Chem.* 3:151–158 (1984).
151. Ingersoll, C. G., F. J. Dwyer, and T. W. May. "Toxicity of Inorganic and Organic Selenium to *Daphnia magna* (Cladocera) and *Chironomus riparius* (Diptera)," *Environ. Toxicol. Chem.* 9:1171–1181 (1990).
152. Burton, G. A., Jr. "Assessing Toxicity of Freshwater Sediments," *Environ. Toxicol. Chem.* 10: 1585–1627 (1991).