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REVIEW AND SYNTHESIS OF BIOASSESSMENT METHODOLOGIES FOR FRESHWATER CONTAMINATED SEDIMENTS

by

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PREFACE

Section 118(c)(3) of The Water Quality Act of 1987 authorized the US Environmental Protection Agency's (EPA) Great Lakes National Program Office (GLNPO) to coordinate and conduct a 5-year study and demonstration project relating to the control and removal of toxic substances in the Great Lakes with emphasis on removal of contaminants associated with in-place bottom sediments. This project is called the Assessment and Remediation of Contaminated Sediments (ARCS) program.

Because the US Army Corps of Engineers has expertise in dredging and management of contaminated sediments, the Corps was asked to be involved in the ARCS program. Participation was formalized at the national level via a memorandum agreement between the Assistant Secretary of the Army for Civil Works and the EPA Assistant Administrator for Water (28 Jan 1989) and at the local level via an interagency agreement between the US Army Engineer (USAE) Division, North Central (NCD) and GLNPO (22 Sep 1988). The USAE Waterways Experiment Station (WES) provided technical guidance to NCD.

This report represents the first published technical guidance from WES to NCD as part of the ARCS program. It is a review and compilation of methods used to determine the potential adverse impact of contaminated bottom sediments on aquatic biota. This information was designed to provide initial guidance for developing methods to assess the biological effects of in-place contaminated sediments.

The review was carried out at the Environmental Laboratory (EL) of WES, Vicksburg, MS, from November 1988 to March 1989 by Dr. Tom M. Dillon and Ms. Alfreda B. Gibson of the Ecosystem Research and Simulation Division (ERSD), EL. The work was performed under the general supervision of Dr. Lloyd H. Saunders, Group Leader, Contaminant Mobility and Regulatory Criteria Group. The Chief of ERSD was Mr. Donald Robey and Chief of EL was Dr. John Harrison.

COL Larry B. Fulton, EN, was Commander and Director of WES. Technical Director was Dr. Robert W. Whalin.

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REVIEW AND SYNTHESIS OF BIOASSESSMENT METHODOLOGIES
FOR FRESHWATER CONTAMINATED SEDIMENTS

PART I: INTRODUCTION

Background

1. Section 118(c)(3) of The Water Quality Act of 1987 directs the Great Lakes National Program Office (GLNPO) of the US Environmental Protection Agency (USEPA) to conduct a study of techniques for the evaluation, control, and removal of contaminated bottom sediments. Field demonstrations of these techniques will be conducted at the five priority Great Lakes Areas of Concern (AOC); Ashtabula River, Ohio, Buffalo River, New York, Grand Calumet River, Indiana, Saginaw Bay, Michigan, and Sheboygan Harbor, Wisconsin.

2. A necessary first step in this process is the identification of methods for assessing the potential biological impact of in-place contaminated bottom sediments. A number of evaluation procedures have been proposed and/or developed by industry, academia, and state and Federal governments. These techniques vary greatly depending on the stage of development, level of biological organization, logistical requirements, and ability to interpret pertinent ecology. Prudent use of Section 118 program resources suggests that well-developed, proven techniques should be considered in designing and implementing a program.

Objectives

3. This report is a review and synthesis of bioassessment techniques that have been used by others for evaluating the potential biological impacts of contaminated bottom sediments. Promising methodologies are identified and a generic tiered-testing protocol is developed.

PART II: APPROACH

Levels of Biological Organization

4. Bioassessment of contaminated sediment can be carried out at several different levels of biological organization. In order of increasing complexity they are: molecular, cellular, tissue, organismic (whole animal), population, and community. When a perturbation occurs at any level of biological organization, mechanistic explanations can generally be found at lower levels of complexity while ecological implications are usually found at higher levels of complexity (Bayne 1985). In the aquatic environment, the main focus of environmental protection is the maintenance of healthy, viable populations of organisms. Unfortunately, forecasting potential impacts at this level of biological complexity is difficult and predictive capabilities are not well-developed. Bioassessments at lower levels of complexity (molecular-tissue) are usually more sensitive but their ecological relevance is often unclear. For these reasons, a surrogate toxicological approach utilizing organismic endpoints is often employed (Chapman 1983; Capuzzo, Moore, and Widdows 1988). This approach represents a propitious balance between response sensitivity in the measurement and ecological relevance in data interpretation (Figure 1). It is also logistically attractive since most contract and research facilities in the United States are capable of conducting these types of whole-animal bioassessments. Therefore, to meet the goals of the Section 118(3)(c) program, this review has focused on papers in the literature that utilize the surrogate toxicological bioassessment approach for evaluating in-place contaminated sediments.

Literature Search

5. A broad sweep of the published literature was initiated by using a range of geographic, biological, and physico-chemical search terms with several on-line search services such as Bibliographic Retrieval Services (BRS), DIALOG, SCISEARCH, National Library of Medicine (NLM), ORBIT, and QUESTEL. Coverage included peer-reviewed journals such as Great Lakes Research as well as the "gray literature" (e.g., National Technical Information Service). This initial search yielded nearly 500 potentially useful citations. After careful

LEVELS OF BIOLOGICAL ORGANIZATION

	BIOCHEMICAL	ORGANISMIC	POPULATIONS	COMMUNITIES
TYPICAL RESPONSE PARAMETERS	BLOOD CONSTITUENTS	BEHAVIOR	ENERGY FLOW	ENERGY FLOW
	PROXIMATE COMPOSITION	MORPHOLOGY	NUTRIENT CYCLING	NUTRIENT CYCLING
	ADENYLATE POOLS	OSMOREGULATION	INTRASPECIFIC INTERACTIONS	INTRASPECIFIC INTERACTIONS
	NUCLEIC ACIDS	METABOLISM	ABUNDANCE	ABUNDANCE
	MEMBRANES	GROWTH		DIVERSITY
	ENZYMES	REPRODUCTION		

RESPONSE
TIME

MINUTES TO HOURS

HOURS TO DAYS

WEEKS TO YEARS

MONTHS TO DECADES

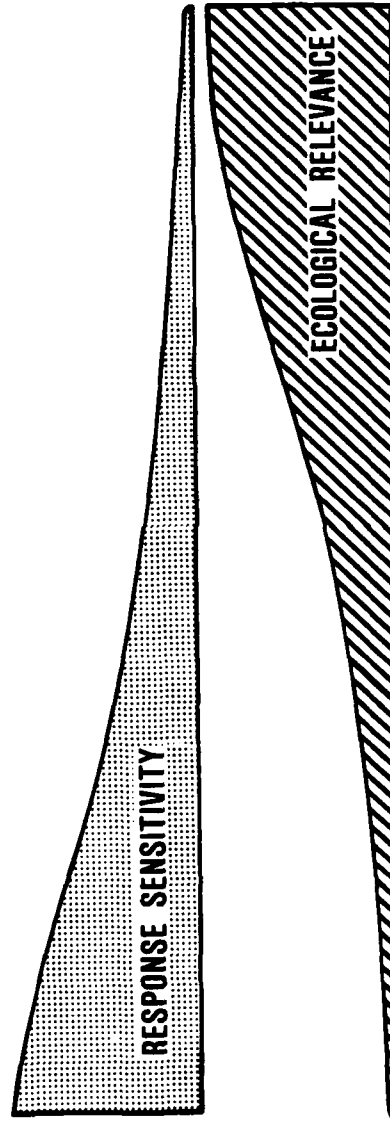


Figure 1. Levels of biological organization

inspection, approximately 200 were deemed applicable for this review. Citations not already on file were obtained and entered into onsite user files. Publications were then individually reviewed for content and technical quality.

6. Scientists and active researchers in the field of sediment bioassessment often participate in workshops and seminars. Literature resulting from these activities can be a very valuable source of information since it often reflects "the best of the best" from the scientific community. Much of this literature also contains any technical consensus agreements arrived at by experts in the field. Therefore, as part of this literature search, current activities in the following areas were reviewed:

- a. American Society for Testing and Materials
- b. International Joint Commission
- c. US Environmental Protection Agency
- d. US Army Corps of Engineers

7. This literature review was restricted to activities and publications dealing with the freshwater environment only. It is acknowledged that some information from the marine and estuarine literature may be conceptually applicable to the generic subject of bioassessment. However, the practical aspect of combining that information with the freshwater literature (e.g., species selection) and formulating specific recommendations for GLNPO precludes its inclusion.

PART III: RESULTS AND DISCUSSION

Bioaccumulation Potential

8. The bioassessment process consists of two elements; (a) the determination of bioaccumulation potential, and (b) an evaluation of biological effects. The majority of literature reviewed for this report emphasizes the bioaccumulation of contaminants from sediment (compiled in Appendix A). The biological effects of freshwater sediment were examined in a smaller number of papers. This dominance of bioaccumulation studies has been noted in previous reviews (Leland, Copenhaver, and Wilkes 1975; DePinto, Young, and Martin 1983; Olsen 1984; Lathrop and Davis 1986; Davis, Denbow, and Lathrop 1987; Davis and Denbow 1988).

9. A determination of contaminant bioavailability is an important and requisite first step in the bioassessment process because from a toxicological perspective, "there are no toxic substances, only toxic doses" [Paracelsus ca. 1540]. In other words, before contaminated sediments can have an effect, the organism must experience a dose. Most environmental contaminants are associated very closely with the sediment matrix and not readily available for uptake (Neff 1984). This is especially true for some of the more toxic non-polar compounds that are very hydrophobic. McFarland and Clarke (1987) have developed a two-tiered process for determining the bioaccumulation potential of these neutral organic contaminants. The first tier estimates the equilibrium tissue concentration relative to the sediment using fugacity theory, data from sediment chemistry, and representative lipid concentrations in biota. If this screening exercise indicates bioaccumulation beyond levels of concern, empirical determinations of bioaccumulation potential may be carried out in the second tier. Time-sequenced samples are taken during laboratory experiments to estimate steady-state tissue concentrations. Long-term exposures may also be carried out to verify predictions of steady-state concentrations.

Contaminant-Specific Biological Effects

10. Papers that did report the biological effects of freshwater sediments focused primarily on a specific contaminant or class of

contaminants. These investigations employed either a spiking procedure or attempted to correlate concentrations of sediment contaminants with observed biological effects. The sediment spiking approach allows one to evaluate the fate and effects of individual contaminants in the sediment matrix under controlled conditions (for representative examples see Muir, Townsend, and Lockhart (1983); Cairns et al. (1984); Adams, Kimerle, and Mosher (1985); Adams et al. (1986); Nebeker et al. (1986); Swindoll and Applehans (1987); Wood et al. (1987); Hammer, Merkowsky, and Huang (1988); Schuytema et al. (1988). Care must be taken, however, when interpreting the results of sediment spiking experiments especially if one is attempting to simulate natural conditions.

11. Bioaccumulation from spiked sediments can usually be resolved into two phases; a rapid phase lasting hours to days and a slow phase lasting weeks to months (Muir et al. 1983, Wood et al. 1987). This pattern closely mirrors reported desorption curves for a variety of contaminants (Karickhoff 1980; Nyffeler, Li, and Santschi 1984; Tessier et al. 1984; Karickhoff and Morris 1985). Observations on biological effects made during the initial desorption/bioaccumulation phase would not approximate chronic exposure conditions with natural sediments. Complicating the issue is the fact that spiking methods vary greatly and post-spike equilibration times range from a few hours (Hall et al. 1986) to several weeks (Oliver 1987).

12. The other chemical-specific publications dealing with biological effects relate sediment toxicity to concentrations of individual contaminants in sediment. Attempts to identify these correlations have experienced varying degrees of success (Wentsel, McIntosh, and Atchison 1977; Laskowski-Hoke and Prater 1981; West et al. 1986; Fabacher et al. 1988). Generating significant correlations, however, does not mean a causal relationship has been identified. Natural sediments typically contain a wide variety of contaminants; some known, some unknown, and some extremely difficult to detect analytically. This is especially true for sediments from the Great Lakes AOC's, many of which are located in highly industrialized waterways. Sediments from these locations contain hundreds of anthropogenic substances.

13. Projecting cause-and-effect relationships based on an inventory of sediment chemistry ignores several important phenomena:

- a. Contaminant-contaminant interactions (e.g., synergism)
- b. Variations in contaminant activity (e.g., bioavailability)
- c. Chemically undetected toxic substances

The furan and dioxin congeners, especially 2,3,7,8-TCDD, are very good examples of the latter possibility. These compounds are extremely toxic, difficult to analyze, and found at very low concentrations in environmental samples.

Generic Bioassessment Techniques for Freshwater Sediments

14. One way to avoid some of the problems associated with the chemical-by-chemical approach is to consider the contaminated sediment as a single entity. In other words, assess the aggregate effect of all its constituents. Survival in acute or short-term sediment exposures is the most common means of measuring this aggregate effect. The opposite of survival, mortality, represents a clear, albeit severe, potential for adverse environmental impact. The interpretation of acute toxicity bioassays is straightforward. Results are binary; the test animals either survived or they did not.

15. In addition to survival, chronic or sublethal effects on aquatic animals exposed to contaminated sediment have been evaluated, although much less frequently. There are several reasons why chronic or sublethal effects are of interest. As discussed earlier, many environmental contaminants are tightly bound to the sediment matrix and bioaccumulation may occur very slowly. Some compounds may not be acutely toxic but rather exert subtle sublethal effects only after prolonged or chronic exposure. Since the ultimate goal in protecting any biological resource is the maintenance of viable populations, growth and reproduction are two of the more desirable chronic or sublethal end points (Bayne et al. 1980).

16. Investigations were identified in this literature review that have used survival, growth, and reproduction to evaluate freshwater sediment regardless of its contaminant composition. These references have been grouped below according to individual test species. Descriptive comments germane to each test are also provided.

Bioassay Test Organisms for Evaluating Freshwater Sediment

Daphnia magna (cladoceran)

Availability:

Culture or field-collected

Bioaccumulation Potential:

Generally feasible only for single compounds

Biological Effects:

Acute lethality (24-96 hr)

Survival, growth, and reproduction in full or partial life cycle exposures

For detailed methods see: Prater and Anderson (1977), Hoke and Prater (1980), Malueg et al. (1983, 1984), Nebeker et al. (1984), LeBlanc and Surprenant (1985), Ziegenfuss and Adams (1985), Adams et al. (1986)

Comments: More sensitive than most other test species. Primarily nektonic and perhaps epibenthic. Not intimately associated with the benthic environment.

Chironomus tentans (midge)

Availability:

Culture or field-collected

Bioaccumulation Potential:

Generally feasible only for single compounds

Biological Effects:

Acute lethality (24-96 hr)

Survival and growth in partial life cycle exposures

Adult emergence

Behavioral avoidance

For detailed methods see: Mosher and Adams (1982); Mosher, Kimerle, and Adams (1982); Nebeker et al. (1984); Ziegenfuss and Adams (1985); Wentzel et al. (1977); Wentzel, McIntosh, and Atchison (1977); Wentzel, McIntosh, and McCafferty (1978); Adams et al. (1986)

Comments: Larval growth occurs in four instars, each lasting approximately one week. First instars typically show poor survival in the laboratory so all tests are initiated with second-instar larvae. Survival and growth are measured after reaching the fourth instar. Normal adult emergence is usually very low; less than 60 percent. *C. riparius* has also been suggested due to its larger size and slightly faster developmental time. It is also more common in the Great Lakes than *C. tentans*. However, methods for this congener are not as well-developed.

Hexagenia limbata (mayfly)

Availability:

Field-collected

Bioaccumulation Potential:

Generally feasible only for single compounds

Biological Effects:

Acute lethality (24-96 hr)

Growth

Adult emergence

For detailed methods see: Prater and Anderson (1977), Hoke and Prater (1980), Landrum et al. (1983), Malueg et al. (1983, 1984), Nebeker et al. (1984), Landrum and Poore (1988)

Comments: Long generation times and variable growth rates make laboratory cultures difficult. Cannibalism and high control mortality observed in larger animals.

Asellus communis (isopod)

Availability:

Field-collected

Bioaccumulation Potential:

Useful with natural sediments

Biological Effects:

Acute lethality (24-96 hr)

For detailed methods see: Prater and Anderson (1977), Lewis and McIntosh (1986)

Comments: Paucity of background information and no culturing techniques.

Hyalella azteca (amphipod)

Availability:

Culture or field-collected

Bioaccumulation Potential:

Generally feasible only for single compounds

Biological Effects:

Acute lethality (24-96 hr)

Survival and growth in partial life cycle exposures

For detailed methods see: Landrum and Scavia (1983), Nebeker et al. (1984), Nebeker and Miller (1988)

Comments: The life cycle is approximately 27 days. Easy to culture but determination of reproductive success is difficult due to non-quantitative recovery of young amphipods while sieving sediment.

Gammarus lacustris (amphipod)

Availability:

Culture or field-collected

Bioaccumulation Potential:

Useful with natural sediments

Biological Effects:

Acute lethality (24-96 hr)

For detailed methods see: Nebeker et al. (1984)

Comments: Cannibalism and high control mortality observed in larger animals.

Pontoporeia hoyi (amphipod)

Availability:

Field-collected

Bioaccumulation Potential:

Generally feasible only for single compounds

Biological Effects:

Acute lethality (24-96 hr)

For detailed methods see: Gannon and Beeton (1971), Landrum et al. (1983), Landrum (1988)

Comments: Indigenous to the Great Lakes and an important component of the benthic ecosystem. Difficult to collect and must be maintained under cold-water conditions.

Stylodrilus heringianus (oligochaete)

Availability:

Field-collected

Bioaccumulation Potential:

Generally feasible only for single compounds

Biological Effects:

Acute lethality (24-96 hr)

Burrowing activity

For detailed methods see: Keilty, White, and Landrum (1988), White and Keilty (1988)

Comments: Indigenous to the Great Lakes and an important component of the benthic ecosystem. Not believed to be very sensitive to environmental contaminants.

Pimephales promelas (fathead minnow)

Availability:

Culture or field-collected

Bioaccumulation Potential:

Useful with natural sediments

Biological Effects:

Acute lethality (24-96 hr)

Survival, growth, and reproduction in full or partial life cycle exposures

For detailed methods see: Mac et al. (1984), Dillon (1988), Hoke and Prater (1980), LeBlanc and Surprenant (1985), Prater and Anderson (1977)

Comments: Large size and high lipid content facilitate bioaccumulation studies.

Institutional Bioassessment Techniques For Freshwater Sediments

17. As discussed in the Introduction, many institutional programs have adopted the surrogate toxicological approach for evaluating freshwater sediments. This approach, which extrapolates from laboratory observations with whole animals to potential impacts on feral populations in the field, emphasizes effects on survival, growth, and reproduction. It has the added benefit of being understood by the general public. This facilitates communication of technical findings to a nontechnical audience, a responsibility shared by many institutional programs.

18. In the course of this review, it became apparent that some institutional programs with divergent mission responsibilities utilize very similar bioassessment techniques for freshwater sediment. It is therefore reasonable to conclude that similarities found across such diverse groups probably represent a sound technical consensus of the scientific community. Activities in the following groups were reviewed.

a. American Society for Testing and Materials

b. International Joint Commission

c. US Environmental Protection Agency

d. US Army Corps of Engineers

American Society for Testing and Materials (ASTM)

19. The primary goal of ASTM is to foster the development of technically sound standard methods and materials. The Sediment Toxicology Subcommittee (E47.03) is one of the newest work groups in ASTM. With 225 members, it is also the largest subcommittee in ASTM. This size indicates a widespread interest in the development of standardized sediment-testing methods. This subcommittee is chaired by Dr. Chris G. Ingersoll of the US Fish and Wildlife Service (USFWS) in Columbia, MO.

20. Separate task groups were formed in the Sediment Toxicity Subcommittee to address four issues;

- a. Sediment collection, storage, characterization, and manipulation
- b. Experimental designs for sediment bioassays
- c. Solid phase sediment bioassays to assess toxicity
- d. Solid phase sediment bioassays to assess bioaccumulation potential

21. A draft guidance document for conducting solid phase toxicity tests with freshwater sediments has been submitted to the Subcommittee for discussion and balloting (Nelson, Ingersoll, and Dwyer, in preparation). This is a generic "how-to" document with a discussion on topics such as statistical and experimental design, necessary equipment, potential non-treatment effects, and safety considerations. The authors recommend three test species; *Hyalella azteca*, *Chironomus tentans*, and *Chironomus riparius*. Detailed materials and methods specific to each organism are provided in three separate appendixes. Brief summaries of test conditions for each species are given below.

- a. *Hyalella azteca*. Provide exposure at 20° C in 1-l beakers or 20-l aquaria for <10 days (short-term partial life cycle) or >10 days (long-term partial life cycle). Monitor survival and growth. Reproduction can be evaluated if test duration exceeds 30 days.
- b. *Chironomus tentans*. Provide exposure at 20° to 23° C in 2-l or 20-l aquaria for 10-14 days when measuring larval survival and growth and 25 days when measuring adult emergence. Exposures can also be carried out in 50-ml centrifuge tubes under some experimental designs.
- c. *Chironomus riparius*. Conditions are generally similar to those for *Chironomus tentans* although test development for this species is not as well developed. The advantages of *C. riparius*

are its larger size and slightly faster development time. It is also more common in the Great Lakes than *C. tentans*.

22. ASTM guidance on how to assess bioaccumulation potential of contaminants in freshwater sediments is in its formative state. Discussion outlines indicate that at least one of the recommended test organisms will be the fathead minnow, *Pimephales promelas*.

23. Three different phases of sediment can be evaluated; solid phase, suspended particulate phase, and elutriate. The decision on which phase to test is dependent on the part of the environment in which potential impacts may occur. The selection of which phase to test can also be driven by a specific research hypothesis. Most of the Sediment Subcommittee's concerns have focused on the solid-phase testing of sediments. Laboratory research and field observations indicate that if significant impacts from contaminated sediments do occur, they are usually associated with the benthic environment (Engler 1980). In addition to ASTM, the USFWS, USEPA, and the US Army Corps of Engineers have recommended that primary emphasis be placed on solid phase testing unless there is some reason to believe impacts in the water column may occur (Saucier et al. 1978; Mac et al. 1984; Seelye, Hesselberg, and Mac 1982; Seelye and Mac 1984).

International Joint Commission (IJC)

24. The IJC is a long-standing bilateral commission composed of representatives from Canada and the United States. The United States and Canada share many common waterways located primarily in and around the Great Lakes. One of the common areas of interest is the pollution of these waterways and specifically the presence of environmental contaminants in bottom sediments. The Sediment Subcommittee of the IJC was formed to address this issue. It has recently reviewed a variety of procedures for the bioassessment of contaminated sediments in the Great Lakes (IJC 1988). The report recommends a tiered approach that includes the following six laboratory bioassessment techniques.

- a. Microtox. In this assay, luminescent bacteria (*Photobacterium phosphoreum*) are exposed for a short period of time (15-30 min is typical) to an extract or aqueous sample. A decrease in luminescence is interpreted as a sign of toxicity. The primary advantage of this assay is its quickness and low cost per sample. The report notes, however, that this bacterial assay is less sensitive to mercury and cadmium than *Daphnia magna* and that it may be insensitive to chlorinated hydrocarbons such as polychlorinated biphenyls (PCB's). The Microtox assay may be useful in screening large numbers of

samples when broad overall assessments regarding groups of samples are being made. It may be less useful than more traditional and reliable screening bioassays (e.g., *Daphnia magna*) when decisions regarding individual samples are needed.

- b. Algal photosynthesis. Photosynthesis is the autotrophic synthesis of organic material by plants using light as an energy source. The fixation of ^{14}C by algae is utilized in this assay as a surrogate for photosynthesis. Either laboratory cultures of algae (e.g., *Selenastrum capricornutum*) or field-collected samples are tested. This assay is used to evaluate potential water column impacts. Fisheries and Oceans Canada utilizes the algal photosynthesis assay extensively.
- c. Life cycle bioassays with *Daphnia magna*. See earlier discussions regarding this organism.
- d. Chronic benthic invertebrate bioassays. Three test species are recommended: *Chironomus tentans*, *Hyalella azteca*, and *Hexagenia limbata*. See earlier discussions regarding these organisms.
- e. Bioaccumulation with *Pimephales promelas*. See earlier discussions regarding this organism.
- f. Ames test. Fish from areas containing contaminated freshwater sediment (especially polycyclic aromatic hydrocarbons) have been observed with various internal tumors and epidermal papillomas. Neoplasia has been experimentally induced in freshwater fish exposed to contaminated sediment in the laboratory. The somatic mutation theory requires the mutation of genetic material prior to carcinogenesis. Therefore, an assay that identifies mutagenic activity would indicate a potential for carcinogenicity. The Ames test, developed for mammalian toxicology, is the most commonly used assay for mutagenicity. Specific strains of *Salmonella* are exposed to sediment extracts and the number of revertants (mutagenicity) is observed. Microsomal fractions from rat liver or fish liver can be added to these extracts to detect mutagens that require activation. In mammals, the induction of cancer correlates strongly and positively with results of the Ames test. However, it has been criticized for showing no response in many instances where subsequent tumors were detected (Tennant 1987).

25. The IJC report acknowledges that much research and development is required in the area of genotoxicity. It proposes two approaches. In one, a chemical-by-chemical determination of mutagenicity would be carried out with selected contaminants. Results would then be compared to bulk sediment concentrations. The other approach is more direct. Using the small fish carcinogenesis model (Hawkins, Overstreet, and Walker 1988), in vivo fish bioassays would be carried out with the test sediment. Fish would be monitored over time for the presence or absence of neoplasia.

26. The current literature review discovered additional citations not contained in the IJC report in which the Ames test is used to evaluate the mutagenicity of freshwater sediments (Allen, Noll, and Nelson 1983; Samoiloff et al. 1983; West et al. 1986; Oishi and Takahashi 1987). Two recent reports suggest another genotoxic end point (unscheduled DNA synthesis) may also be useful and should receive further attention (Fabacher et al. 1988, West et al. 1988).

US Environmental Protection
Agency (USEPA)

27. In April 1988, Region V USEPA published its draft guidance for sampling and testing of freshwater sediments. This guidance uses bulk chemistry data to classify sediments into three categories; Type I (probably unpolluted), Type III (probably polluted), and Type II (other). Most of the document is devoted to describing this classification scheme and to sediment sampling procedures. Three bioassessment protocols recommended by Region V in this document are shown below. See earlier discussions regarding details of organism-specific procedures.

- a. Elutriate bioassays with cladocerans. *Daphnia magna* is recommended.
- b. Solid phase sublethal sediment bioassays with either the midge, *Chironomus tentans*, or the amphipod, *Hyalella azteca*.
- c. Solid phase bioaccumulation studies with the fathead minnow, *Pimephales promelas*.

US Army Corps of Engineers (USACE)

28. In 1985, the US Army Engineer District, St. Paul, cosponsored a workshop with the Wisconsin Department of Natural Resources (DNR) for the purpose of identifying bioassessment methods for the regulatory evaluation of freshwater sediment. Detailed proceedings of this workshop and supporting technical documentation can be found in Dillon and Gibson (1986). Major consensus agreements reached by the workshop's participants are summarized below.

- a. Tiered or hierarchical testing represents a technically sound process for evaluating freshwater sediments.
- b. Inventories of chemicals in sediment give no indication of biological effects but are useful in deciding if further evaluation is necessary. Chemical inventories can be used to predict bioaccumulation potential of certain contaminants under certain conditions.
- c. Solid phase sediment testing should be emphasized unless there is a reason to believe water column impacts may occur.

- d. Growth and reproduction should be considered the highest priority sublethal end points.
- e. Some sort of oncological assessment is highly desirable. However, no single method could be recommended as having been sufficiently developed for use in a regulatory program.

29. The generic tiered-testing protocol produced by the workshop participants has been more fully developed by the St. Paul District. Working in conjunction with the Wisconsin DNR, this protocol has been tested in the laboratory using sediments from the Great Lakes and Mississippi River (Ward, in preparation). The bioassessment techniques contained in this tiered-testing protocol are summarized below.

- a. Solid phase acute (48-hr) lethality bioassay with *Daphnia magna*.
- b. Solid phase chronic (10-day) bioassay measuring survival, growth, and reproduction in *Daphnia magna*.
- c. Solid phase chronic (14-day) bioassay measuring survival and growth in *Chironomus tentans*.
- d. Solid phase bioaccumulation (30-day) with fathead minnows, *Pimephales promelas*.

PART IV: SUMMARY AND RECOMMENDATIONS

30. Review of the bioassessment methodologies for freshwater contaminated sediments results in the following conclusions:

- a. The bioassessment literature for freshwater sediment is dominated by investigations that evaluate contaminant bioaccumulation and not the biological effects of contaminated sediments.
- b. The majority of investigations that evaluate biological effects have focused on specific contaminants or groups of contaminants.
- c. The chemical-by-chemical approach utilized in many of these bioassessment techniques cannot account for three important phenomena:
 - (1) Contaminant-contaminant interactions (e.g., synergism).
 - (2) Variations in contaminant activity (e.g., bioavailability).
 - (3) Chemically undetected toxic substances (e.g., dioxin).
- d. Several institutional programs with diverse mission responsibilities (ASTM, IJC, USEPA, USACE) are using or considering very similar bioassessment techniques for the evaluation of contaminated freshwater sediments. These similarities strongly suggest that a technical consensus exists in the scientific community regarding appropriate bioassessment techniques. These similarities can be summarized as follows:
 - (1) Tiered testing represents a logical, technically sound approach for evaluating contaminated sediments.
 - (2) Sensitive test species, especially cladocerans such as *Daphnia magna*, should be used in the initial biological screening tiers.
 - (3) Chronic or sublethal testing should include an evaluation of impacts on growth and reproductive success.
 - (4) Recommended test species for evaluating chronic or sublethal impacts are the midge, *Chironomus tentans*, and the amphipod, *Hyalella azteca*.
 - (5) The fathead minnow, *Pimephales promelas*, may be used to evaluate bioaccumulation potential. However, fish, as well as most aquatic animals, can metabolize polycyclic aromatic hydrocarbons. Therefore, parent compounds may not be easily detected in tissues.
 - (6) Bioassessments should emphasize solid phase sediment bioassays unless there is a reason to believe that water column organisms will be seriously impacted.

31. The following protocol is recommended for tiered-testing bioassessment.

a. Tier I. Initial evaluation of existing data.

b. Tier II. Chemical analysis of sediment.

Bioaccumulation potential: Calculation of equilibrium tissue concentrations of neutral organics relative to sediment concentrations.

Biological effects: Screening bioassays.

c. Tier III. Bioaccumulation potential: Empirical determination during laboratory exposures.

Biological effects: Short-term solid phase toxicity tests.

d. Tier IV. Bioaccumulation potential: Steady-state determination and important factors affecting uptake.

Biological Effects: Chronic or sublethal solid phase tests and important factors affecting toxicity.

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**APPENDIX A: BIBLIOGRAPHY OF CONTAMINANT-SPECIFIC FRESHWATER
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